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**Biotechnology of Chiral Sulfoxyphospholipid Production:
A Feasibility Study**

by

Derek J. Hodgson B.Sc.

**A Thesis submitted to the Faculty of Graduate Studies
in partial fulfillment of the requirements for the degree of
Master of Science**

to

The Faculty of Graduate Studies and Research

Department of Chemistry

Carleton University

Ottawa, Canada

April 2002

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**Biotechnology of Chiral Sulfoxyphospholipid Production:
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Chair, Department of Chemistry

Thesis Supervisor

Abstract

The chain length dependence on the enzymatic formation of enantiomerically enriched 9-sulfoxy fatty acids was investigated for eleven 9-thia fatty acid methyl ester substrates, (C_{10} to C_{20}). The optimum fatty acid chain lengths for sulfoxidation, by *S. cerevisiae*, was determined to be C_{15} and C_{16} . Methyl 9-*R*-sulfoxypalmitate, (% ee. > 90 %) was produced by repeated batch culture in 38 % yield, after purification. Characterization of methyl 9-*R*-sulfoxypalmitate was completed using ^1H and ^{13}C NMR.

9'-*R*-9''-*R*-Disulfoxypalmitoyl-3-*sn*-phosphatidylcholine, [cDP(SO)PC], was synthesized via the condensation reaction of 9-*R*-sulfoxypalmitic acid and L-(+)- α -glycerophosphorylcholine, in the presence of dimethyl-4-aminopyridine, (DMAP), and dicyclohexylcarbodiimide, (DCC), in 36 % yield. cDP(SO)PC was characterized via ^1H , ^{13}C NMR and electrospray mass spectrometry. Stereochemical purity of cDP(SO)PC was evaluated via ^{13}C NMR shift reagent experiments, as well as 2-D studies of molecular stacking using a Langmuir balance.

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Abbreviations List

CoA – Coenzyme A

DCC – Dicyclocarbodiimide

DMAP – Dimethyl-4-aminopyridine

dH₂O – Distilled Water

ddH₂O – Deionized Distilled Water

DPPC – Dipalmitoyl-3-*sn*-phosphatidylcholine

DP(S)PC – 9',9''-Dithiapalmitoyl-3-*sn*-phosphatidylcholine

rDP(SO)PC – 9',9''-(*R,S*)-Disulfoxypalmitoyl-3-*sn*-phosphatidylcholine

cDP(SO)PC – 9'-*R*-9''-*R*-Disulfoxypalmitoyl-3-*sn*-phosphatidylcholine

DP(SO₂)PC – 9',9''-Disulfonylpalmitoyl-3-*sn*-phosphatidylcholine

Chapter 1 – Introduction

1.1 Lipids

1.1.1 Background

Historically, biological materials have been subdivided into fats, proteins, carbohydrates, nucleic acids, and minerals. Fats are molecules, made up of two components: glycerol and fatty acids, that can be distinguished from other natural products by their solubility in non-polar solvents, and relative insolubility in water.¹ They are a part of a larger grouping of hydrophobic natural products, that include phospholipids and steroids, called lipids.

Lipids are involved in many biological processes, including the construction of biomembranes, the transduction of cellular signals, and as a source of energy.² The molecules must contain three essential features: a polar head group, one or more hydrophobic tail regions, and a backbone that connects the two.³ Numerous variants in the head and tail groups lead to a tremendous number of compounds that fall into the category of lipids. The backbone of the lipid is used to separate these biomolecules into various subgroups: isoprenoids, phospholipids, sphingolipids, cerimides, fatty acids, triacylglycerols, eicosanoids, waxes, and glycerols.² The most common headgroups associated with these molecules, (choline, ethanolamine, inositol and serine), may render the lipids either charged, uncharged or zwitterionic at physiological pH.²

Figure 1.1.1 Structures of Some Common Phosphoglyceride Headgroups

The presence of the negatively charged phosphate, and the positively charged amine imparts a polar head region, while leaving the phosphoglycerides electrically neutral.

The highly polar head group, and long non-polar acyl chains bestows the characteristic amphipathic nature that is critical in membrane structure.³

1.1.3 Nomenclature of Phospholipids

To correctly assign a name to a phospholipid, one must first look at the glycerol backbone. The addition of a substituent to one of the terminal alkyl groups will result in the *meso* carbon becoming asymmetric, giving the molecule optical activity.

Figure 1.1.3 Glycerol Molecule

A problem that arises in biological systems, with respect to the primary hydroxyl groups, is that they do not undergo identical reactions. The selectivity of enzymes differentiates between the hydroxyls.¹ To bring clarity to this, a system that can distinguish between the first and third carbon was developed by Hirshmann.⁵ The 'stereospecific numbering' requires that a fixed configuration of L-glyceraldehyde, (*S*-glyceraldehyde), is applied to glycerol.

Figure 1.1.4 Stereochemical View of Glycerol

The carbon above the central carbon is assigned as C-1, and the one below is C-3.⁵ The use of stereospecific numbering is indicated by “*sn*” before the root name of the compound, eg. 1,2,3-triacyl-*sn*-glycerol.

A common feature of all phospholipids is the presence of a phosphate group linked in the C-3 position of the *sn*-glycerol backbone.³ The wide variety in phospholipid structure comes from the numerous tail groups that can be attached to the glycerol, as well as the various types of head groups. The tail groups are located on the C-1 and/or C-2 carbon of the glycerol chain and are described in the prefix to the name of the compound.

Figure 1.1.5 1,2-Diacyl-*sn*-glycero-3-phosphate

As mentioned earlier, glycerophospholipids vary in derivatives of the phosphate bond, and are named in accordance to the substituent; e.g. a choline derivative is 1,2-diacyl-*sn*-glycero-3-phosphocholine. A shorthand notation of nomenclature, commonly used for glycerolphospholipids, involves the use of phosphatidyl to denote the phosphatidate portion of the molecule.¹

Figure 1.1.6 1,2-Diacyl-3-*sn*-phosphatidylcholine

1.1.4 Bilayers

Phospholipid bilayers are ubiquitous to living organisms as important structural components of biological membranes in which proteins are embedded.⁶ Initial studies in the flexibility of membranes lead to the introduction of the well known fluid-mosaic model, (Figure 1.1.7).⁷

Figure 1.1.7 Fluid Mosaic Model (Adapted From Reference 4)

The fluidity of a certain cell membrane is dependent on the degree of desaturation in the fatty acid tail groups that are packed together. It is of great importance that an organism is capable of maintaining a specific fluidity to regulate membrane-mediated

processes.⁸ The saturated chain gives a tight packing formation, thereby optimizing their van der Waals interactions, (Figure 1.1.8).³ Membrane lipids commonly contain one saturated and one unsaturated fatty acid, which keeps the majority of membrane lipids in the fluid state at physiological temperatures, (Figure 1.1.9).³

Figure 1.1.8 Packing Effects of Saturated Fatty Acid Sidechains
(Adapted From Reference 3)

Figure 1.1.9 Packing Effects of Unsaturated Fatty Acid Sidechains
(Adapted From Reference 3)

1.2 Applications

The medical field has taken an interest in potential applications of phospholipids, in biomineralization and biomaterials research. Biomineralization is a process that occurs in many species for the specific delivery of minerals in the formation of bone, tooth enamel, and the exoskeleton.² A biomaterial can be defined as any material, natural or synthetic, that is used in the repair, replacement, reconstruction, or treatment of diseased or traumatized tissues.² Biomaterial research includes the use of lipid vesicles as a delivery vehicle for drugs,⁹ therapeutic agents,¹⁰ and macromolecules,¹¹ to specific organs, tissues, or cells.

Phospholipids also have the ability to self assemble into a monolayer at the air water interface, thus, providing an opportunity to increase fundamental understanding of self-organization, structure-property relationships, and interfacial phenomena.¹² The overall ability to change the head and tail groups, as well as the stability of self assembled monolayers, (SAMs), give possible applications in corrosion protection and wear prevention of electro-optic devices.¹²

1.3 Thin Films

1.3.1 Langmuir Films

The groundbreaking work of Pockels and Rayleigh in the 1890's established the idea that amphipathic molecules, which are almost insoluble in aqueous media, can form monomolecular layers at the liquid-air interface.¹³ In the early 1900's, Irving Langmuir took an interest in the thin film area, and developed the Langmuir trough balance, (Figure 1.3.1).¹⁴ The trough is a surface balance that contains a movable float separating a clean

water surface from an area covered with a film; the deflection of the float then provides a direct measure of the forces involved.¹⁵

Figure 1.3.1 Langmuir Trough (Adapted From Reference 13)

The technique for acquiring Langmuir films at the air/water interface involves the use of a shallow rectangular Teflon-lined pan filled with deionized water. One or two moving barriers are used to change the area of the water surface on which the sample has been deposited. As this area changes, the surface pressure, π , of the water is monitored. The spreading of the monolayer on the aqueous subphase is completed using an organic solvent. Selection of the solvent used is based on a number of criteria: It must dissolve an adequate quantity of the monolayer material, it cannot react chemically with the material, or dissolve in the subphase, and it must evaporate within a reasonable time period.¹⁶ It is important that the solvent does not evaporate too quickly due to the need for an accurate quantification of concentration. The monolayer will spread evenly throughout the apparatus, causing a unimolecular film. The uniformly spaced molecules will compact to a thin film as the area available decreases, (Figure 1.3.2).¹⁷

**Figure 1.3.2 Monolayer of Molecules In Two Dimensions:
Gas (top) and Solid (bottom)
(Adapted From Reference 17)**

The surface pressure of the thin film is defined as the difference between the surface tension of the 'clean' water phase, (γ_o), and the surface tension of water altered by a sample molecule, (γ), in the following equation:¹⁵

$$\pi = \gamma_o - \gamma$$

The absolute measurement of the surface pressure is made using a Wilhelmy plate attached to a sensitive balance. The mass of the plate will vary with the downwards surface tension of the monolayer.¹⁶ Thus, the surface tension difference between the clean and contaminated water will result in the surface pressure value. As molecules become more packed, the air/water surface tension decreases, causing the surface pressure to increase.

1.3.2 Isotherms

A plot of surface pressure vs. area results in a surface pressure/area isotherm that contains information on the stability of the monolayer. Figure 1.3.3 exhibits a 'gas',

'liquid' and 'solid' phase. The 'gas' phase of the isotherm ideally shows no interactions between the molecules on the surface, thus giving a low surface pressure. As the area decreases, the individual molecules will start to 'feel' neighbouring effects, which increases the surface pressure.¹⁷ The effects of the neighbours cause the hydrophobic end to leave the water phase and stick out of the solution. The 'liquid' phase is a result of the first phase transition, known as the liquid-expanded state. Continued decrease in area will compress the film through a second transition state, known as the liquid-condensed state, to the 'solid' phase.¹⁷ The close 'packing' of the phospholipids, in the 'solid' phase, causes all of the hydrophobic tails to emerge from the subphase.

Figure 1.3.3 Sample Isotherm for Stearic Acid
(Adapted From Reference 17)

Isotherms can be used in determining the stacking ability of a given molecule, through analysis of the area per molecule at the phase transition. The ease with which a film can compress is characteristic of its stacking ability, ie. the better the stacking

ability, the less area per molecule is needed in the liquid-expanded phase.¹⁸ Another measure of stability can be established by examining the phase transition temperatures of the molecules, via differential scanning calorimetry, (DSC). The thermally induced main phase transition of lipid bilayers, (T_m), from the ordered gel phase to the fluid-like liquid-crystalline phase, is governed by the structure of the lipid acyl chain.¹⁹ An improvement in the 'stacking' ability of the lipid will be exhibited by an increase in T_m .

1.4 Modified Phospholipids

1.4.1 Stability of Phospholipids Bearing Tail Group Substituents

An increase in the stabilization of phospholipids is of interest for the prospects of using these biomolecules as delivery agents in the body,^{9,10,11} as well as corrosion protection and wear prevention of electro-optic devices.¹² The phase transition from the gel phase to the liquid-crystalline phase is induced through chain disorder of the fatty acid. This disarrangement of the acyl chains initiates at the chain terminus and propagates towards the centre with the increase in temperature.²⁰ Order parameter studies have established that the side chain is relatively ordered until the midway point of the acyl chain, when the molecular motions increase rapidly and uniformly.^{21,22} Stability of the phospholipid may be gained through the addition of an order inducing moiety in the latter half of the acyl chain.

The introduction of a methyl substituent in the chain has an effect on the packing of the phospholipid. Menger *et al*, determined the effects of a substituted methyl group in the fourth, eighth, twelfth, and sixteenth carbon of the distearoyl-3-*sn*-phosphatidylcholine.¹⁸ Analysis of the isotherm of the sidechains, (Figure 1.4.1), showed

that the area per molecule was greater for the methyl substituted acids than that of stearic acid, thus, the methyl substitution “expanded” the films.

Figure 1.4.1 Pressure-area Isotherm for (A) Stearic Acid (B) 16-Methylstearic Acid (C) 12-Methylstearic Acid (D) 4-Methylstearic Acid (E) 8-Methylstearic Acid (Adapted From Reference 18)

The addition of the methyl substituents had a destabilizing effect on the packing of the tail group of the phospholipids. This could be foreseen due to the addition of the bulky group that contains no possible stabilizing effects. The introduction of a polar moiety into the acyl chain may be able to induce dipole-dipole interactions of the neighbouring groups, thus enhancing the stacking of the phospholipids.

1.4.2 Sulfoxy Phospholipids

Lennox *et al.* has studied the effects of introducing the sulfoxide and sulfone moieties to the hydrophobic tail of distearyl-3-*sn*-phosphatidylcholine, (DSPC). Their work resulted in an increase of the T_m by 13.9 °C, for the sulfone substituent in the ninth

position.²³ The thermal stability was enhanced by the cross-linking of the dipoles, which was expected. However, the introduction of the sulfoxide moiety resulted in a decrease in the T_m by 9.2°C.²³ This outcome was not as expected due to the comparable dipole moments of the two functional groups; SO_2 4.49 D, SO 3.96D.²⁴ This result was attributed to the lower effective dipole-dipole interaction. The dipoles of the racemate, [Figure 1.4.2 (B)], do not interact as well as those for the sulfone, [Figure 1.4.2 (A)].

Figure 1.4.2 Effects of Dipole-Dipole Stacking

Through the use of an enzyme known as Δ^9 desaturase, it is possible to synthesize the 9-sulfoxy carboxylic acid substrate in an enantiomerically “pure” form. The enantiomerically enriched sulfoxides should have the needed dipole alignment to increase the stability of the phospholipid, [Figure 1.4.2 (C)]. Since the dipole moment of the

sulfoxide and sulfone are similar, one might expect a similar increase in the stability of the phospholipid. However, another factor in 'stacking' ability is the size of the substituent located on the acyl chain. Menger *et al.* have ascertained that larger substituents cause greater perturbations in the stacking.¹⁸ Since there is a difference in size between the sulfur-oxygen moieties, we hypothesize that the stabilizing effect of the enantiomerically enriched sulfoxide will be at least equal, if not greater than that of the sulfone.

1.5 Methods for Synthesis of Enantiomerically Enriched Sulfoxides

There are numerous biocatalytic methods for the preparation of chiral sulfoxides for various uses, ranging from regulation of cholesterol catabolism to antibiotic properties.²⁵ Cyclohexanone monooxygenase, from *Acinetobacter calcoaceticus*,²⁶ chloroperoxidase, from *Caldariomyces fumago*,²⁷ toluene and naphthalene dioxygenase,²⁸ and cytochrome P-450-dependent monooxygenases,²⁹ are isolated enzymes that possess high activity for the production of enantiomerically pure sulfoxides. The asymmetric oxidation of sulfides can also be completed *in vivo* via whole-cell biocatalysts using bacteria,³⁰ as well as fungi.³¹

Formation of enantiomerically "pure" sulfoxides can also be completed via the Anderson procedure.³² This reaction involves treating an optically active sulfinic ester with a Grignard reagent, (Figure 1.5.1), and occurs with an inversion of stereochemistry.³³

Figure 1.5.1 Synthesis of Enantiomerically Enriched Sulfoxides (Anderson Procedure)

None of these procedures fulfilled the need of acquiring enantiomeric purity along with high yield of the enantiomerically enriched fatty acyl sulfoxides. To achieve this goal, use of a yeast Δ^9 desaturase was deemed most appropriate.

1.6 Biological Sulfoxidation

1.6.1 Fatty Acid Desaturases

The biological desaturation of fatty acids is an essential biochemical process for most aerobic lifeforms.³⁴ This classical reaction, first discovered by Bloomfield and Bloch in 1958 in bakers yeast (*Saccharomyces cerevisiae*), involves the oxidation of stearoyl-coenzyme A (CoA) to give oleyl-CoA via a four electron reduction of oxygen to give two molecules of water (Figure 1.6.1).³⁵ Two of the electrons involved come from the *pro-R* hydrogens at the ninth and tenth carbon of the substrate; the other two are provided by the reduced form of nicotinamide adenine dinucleotide (NADH).

Figure 1.6.1 Desaturation of Stearoyl-CoA

The enzyme that is responsible for the desaturation process is known as a Δ^x desaturase. The closest carbon to the carboxylate that is involved in the formation of the double bond is represented as the superscripted number following the delta symbol.³⁶

Unsaturated fatty acids are essential for the proper functioning of many plants and animals. As mentioned earlier, the fluidity of certain cellular membranes is dependent on the degree of desaturation in the fatty acids that are packed together. Saturated fatty acids have long, straight tails that pack well together, whereas the introduction of a *cis* double bond causes a bend, or a kink in the molecule that prevents tight packing.³

1.6.2 Biochemistry of Desaturation

Desaturases fall into two classes; soluble plant enzymes and a widespread membrane bound family. The only plant desaturase that has been characterized in any detail is the soluble Δ^9 desaturase which has been isolated from castor seeds. It has been determined that the soluble enzyme contains a carboxylate bridged diiron active site,

similar to that of methane monooxygenase (MMO).³⁴ The less well characterized membrane bound desaturases are thought to contain a diiron active site that is coordinated to histidine groups and span the lipid bilayer twice.³⁴

Membrane bound desaturases that are present in animals, fungi, and bacteria are specific for the CoA, or, phospholipid derivatives of the fatty acid.¹ The first membrane bound Δ^9 desaturase was studied in *S. cerevisiae*, by Bloomfield and Bloch.³⁸ Their studies showed that oxygen needed to be present in order for the desaturation of the fatty acids to occur. Molecular oxygen is required as an electron acceptor for two pairs of hydrogen molecules, one from NADH, and the other from the fatty acyl-CoA.³⁶ The Δ^9 desaturase consists of three major proteins, (1) NADH-cytochrome b_5 reductase, (2) cytochrome b_5 , and (3) a terminal desaturase component or cyanide-sensitive protein (Figure 1.6.2).³⁶ The cytochrome b_5 reductase transfers the electrons from NADH by means of flavin to cytochrome b_5 .⁶ Cytochrome b_5 is a heme-containing protein in which Fe^{3+} can be reduced to Fe^{2+} .³⁶

Figure 1.6.2 Schematic Representation of the Δ^9 Desaturase
(Adapted From Reference 36)

Soluble desaturases that are found in plants are specific for the acyl carrier protein (ACP) derivative of the fatty acid.³⁹ Desaturation by the soluble Δ^9 desaturase involves the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), NADPH-ferredoxin oxidoreductase, ferredoxin, and oxygen (Figure 1.6.3).⁶

Figure 1.6.3 Electron Transfer Chain for Desaturation

Many studies have been completed on the soluble enzyme. Mössbauer and Raman spectroscopy exhibited the characteristic absorption of an oxo-bridged diiron cluster in the soluble plant stearoyl-ACP desaturase.⁴⁰ An X-ray structure of the Δ^9 desaturase has been obtained from *Ricinus communis* (castor) (Figure 1.6.4). The structure exhibits a tight, crescent shaped hydrophobic substrate binding pocket.³⁴ Computer modeling studies suggest that the ninth carbon of the stearoyl substrate is near the iron atom responsible for initial oxidation, represented by one of the pink spheres.³⁴

Figure 1.6.4 View of the Postulated Substrate Channel in Δ^9 Desaturase
(Adapted From Reference 41)

1.6.3 Stereochemistry of Fatty Acid Desaturation

The desaturation of fatty acids involves the removal of one hydrogen from the C-9 and C-10 position. The first studies regarding the geometry of the double bond (*cis* or *trans*), stereochemistry of hydrogen removal (*syn*- or *anti*-elimination), and the enantioselectivity of hydrogens (*pro-R* or *pro-S*) were completed by Schroepfer and Bloch, in the case of Δ^9 desaturation in *Corynebacterium diphtheriae*.⁴²

The stereochemical study of desaturation was carried out using racemic *erythro*- and *threo*-9,10-dideuterated stearates. Analysis of the methyl oleate extracts by mass spectroscopy demonstrated that mainly the dideuterated product was formed from the *erythro*- precursor, whereas, for the *threo*-precursor, the monodeutero products were formed (Figure 1.6.5).⁴² Thus, a *syn*-elimination process was proposed for the desaturation of stearic acid by the Δ^9 desaturase of *C. diphtheriae*.⁴²

Figure 1.6.5 Olefinic Products from Racemic *erythro*- and *threo*-Dideuterated Labeled Precursors

To determine the enantioselectivity of desaturation, chiral stearates, (*R*)-9-, (*S*)-9-, (*R*)-10-, and (*S*)-10-tritiostearic acids, were synthesized and incubated with *C. diphtheriae*. This study involved the measurement of tritium that was retained in the resulting oleic acid, relative to a ^{14}C -labelled standard.⁴² The results of this experiment indicated that there was a loss of tritium in the (*R*)-9- and (*R*)-10-tritiostearic acid incubations, whereas the (*S*)-9- and (*S*)-10-tritiostearic acids gave oleate which had retained the tritium labels.⁴² These results further prove the removal of the pro-*R* hydrogen in the biocatalytic desaturation.

1.6.4 Mechanism of Fatty Acid Desaturation

Despite the amount of research that has been dedicated to this phenomenon, very little information regarding the mechanistic route for desaturation has been uncovered. Bloch and Schroepfer were the first to show that there was an isotope effect on the removal of the hydrogen from the ninth carbon, but not on the tenth carbon, for the Δ^9 desaturation of stearoyl CoA in *C. diphtheriae*.⁴² This suggested that the insertion of the double bond operates via a non-synchronous mechanism, with the removal of the C-9 hydrogen being the rate-limiting step. In recent studies, Buist *et al* have confirmed a large primary deuterium kinetic isotope effect in the removal of the C-9 hydrogen, whereas the cleavage of the hydrogen on C-10 was essentially insensitive to isotopic substitution, in the case of Δ^9 desaturation as it occurs in *S. cerevisiae*.⁴³

Buist *et al*.³⁴ have proposed a mechanistic scheme that relates desaturation to hydroxylation (the insertion of a hydroxyl group). The proposed scheme involves an

initial rate determining hydrogen removal from C-9 to form a carbon-centered radical followed by a collapse to the olefin in one of three ways (Figure 1.6.6).

Figure 1.6.6 Proposed Mechanistic Pathways for Desaturation

The initial hydrogen abstraction is initiated by a high-valent iron-oxo species to form the radical. This species could collapse into the desired olefin by a one electron oxidation-deprotonation (pathway a), simple disproportionation (pathway b), or by a hydroxylation event, followed by a subsequent Lewis-acid catalyzed dehydration (pathway c).²¹ The hydroxylation event may also occur by the iron-oxo species inserting an oxygen atom into the C-H bond (pathway d).⁴⁴

The mechanistic scheme can be written for the membrane bound as well as the soluble desaturases. Although there are structural differences between the two enzymes, there are several important similarities; the catalytic requirement for iron, inhibition by metal chelators, and the stereoselectivity of the desaturation reaction.⁴⁵ These similarities show reasoning for a common mechanism for desaturation, which may include the requirement for a structurally related catalytic active site.⁴⁵

The determination of the site of initial attack (cryptoregiochemistry) for the desaturation of fatty acids has been the primary focus of Buist *et al*, with extensive research on the membrane bound Δ^9 desaturase that is present in *S. cerevisiae*. Due to the desaturation process being catalyzed by an O_2 -dependant non-heme iron protein, an oxygen trapping experiment was performed, (Figure 1.6.7).⁴⁶

Figure 1.6.7 Proposed Mechanistic Pathway for Sulfoxide Formation

It was found that the sulfoxidation of the C-9 substituted fatty acid was consistently more efficient than the C-10 sulfoxidation.⁴⁶ Thus further solidifying that desaturation is initiated on the ninth carbon.

Buist *et al* determined the enantiomeric excess of bio-formed sulfoxides using the chiral shift reagent, *S*-(+)- α -methoxyphenylacetic acid. The complexation of the carboxylic acid to the sulfoxide functional group, (Figure 1.6.8), induces a downfield shift for the signals of the C-8 and C-10 protons, (H_A), in the 1H NMR spectrum, whereas an upfield chemical shift occurs in the ^{13}C NMR spectrum for C-8 and C-10 resonances.⁴³

Figure 1.6.8 Complexing of Methyl 9-*R*-Sulfoxypalmitate with *S*-(+)- α -Methoxyphenylacetic Acid

Comparison of the ^{13}C NMR spectra, of methyl 9-*R*-sulfoxystearate and methyl 9-sulfoxystearate-*10,10-d*₂, revealed the signal disappearance of the C-10 signal, (δ 52.56 ppm), and assignment of C-8, (δ 52.42 ppm). Addition of the shift reagent to the unlabelled sample resulted in an upfield shift of C-10, (δ 0.85 ppm), and C-8, (δ 0.86 ppm). A similar result was found for C-8 in the isotope labeled sample, (δ 0.88 ppm). Mixing studies of the enantiomerically “pure” methyl 9-*R*-sulfoxystearate-*10,10-d*₂ with a racemic mixture showed a small shoulder, slightly downfield, on the C-8 signal due to the presence of the *S* configuration.⁴⁶

1.7 Research Goals

The overall aim of this project was to investigate the stabilizing effects of the chiral sulfoxide moiety on a phospholipid monolayer. The first goal was to determine an appropriate chain length to give the greatest efficiency of enantiomerically enriched 9-sulfoxide bioformation. Upon determining the ideal chain length, the objective was to couple the enantiomerically enriched sulfoxides to the phospholipid precursor, maintain a high percent enantiomeric excess.

Chapter 2 Results and Discussion

2.1 Experimental Approach

The primary focus of this project was to compare the 2D properties of phosphatidylcholine bearing 9-sulfoxyl (racemic), and 9-sulfoxyl (enantiomerically enriched) side chains. In the first part of this thesis, the optimum fatty acid chain length for the enzymatic formation of an enantiomerically enriched 9-sulfoxy fatty acid was determined. In order to test the chain length dependence, all of the fatty acids in the range of methyl 9-thiadecanoate to methyl 9-thiaeicosanoate were synthesized. The racemic sulfoxide standards were also prepared for all of the chain lengths as a reference source for the compounds. The second part of the thesis involved the synthesis of five phospholipids: dipalmitoyl-3-*sn*-phosphatidylcholine, (DPPC), 9',9''-dithiapalmitoyl-3-*sn*-phosphatidylcholine, [DP(S)PC], 9',9''-(*R,S*)-disulfoxypalmitoyl-3-*sn*-phosphatidylcholine, [rDP(SO)PC], 9'-*R*-9''-*R*-disulfoxypalmitoyl-3-*sn*-phosphatidylcholine, [cDP(SO)PC], and 9',9''-disulfonylpalmitoyl-3-*sn*-phosphatidylcholine, [DP(SO₂)PC]. Preliminary Langmuir film studies were performed on this set of compounds. Future studies, by the Lennox group, (McGill University), will determine the phase transition, T_m , using differential scanning calorimetry, (DSC). Upon obtaining the T_m , more indepth studies as to the stacking ability of the compounds can be completed.

2.2 Synthesis of Substrates and Reference Standards

2.2.1 Thia-Fatty Acids

Methyl 9-thia fatty acids of varying chain lengths, ($C_{10} - C_{20}$), were synthesized via a three-step reaction sequence (Figure 2.2.1) in yields ranging from 45 % to 80 %. 8-Bromooctanoic acid was reacted with thiourea to form 8-mercaptooctanoic acid, which was subsequently deprotonated by *n*-butyl lithium (2.5 equivalents), and reacted with a terminal bromo-alkane to give a 9-thiacarboxylic acid. Esterification of the acid to the methyl ester was carried out using boron trifluoride diethyletherate and methanol. The resulting methyl ester was purified by flash chromatography (silica gel, 2.5 % ethyl acetate/hexane), purity > 99 %, as determined via gas chromatography and mass spectrometry analysis, (GC/MS). The purification of methyl 9-thiadecanoate was completed via acid-base chemistry. The methyl ester of the sulfide and unreacted thiol were separated through the use of a three molar sodium hydroxide solution. The pK_a of water is *ca.* 15.5, whereas the pK_a for the thiol is *ca.* 10.5.⁴⁷ Therefore, the hydroxide anion is able to deprotonate the thiol, thus giving a facile separation of the two compounds. This purification technique could only be used for the C_{10} substrate due to difficulties with emulsions in the extraction of longer chain fatty acids. The structures and purity of all of the compounds were confirmed using proton nuclear magnetic resonance spectroscopy, (1H NMR), ^{13}C NMR, as well as GC/MS.

Figure 2.2.1 Synthesis of Methyl 9-Thia Fatty Acids

Initial thin layer chromatography, (TLC), analysis of the thia fatty acid methyl esters revealed that their R_f value, which is a measure of retention of a certain compound on silica gel, did not vary with chain length. The methyl esters that were produced shared a common R_f value of *ca.* 0.14 using an eluent of 2.5 % ethyl acetate/hexanes, (EtOAc/Hex). This result demonstrates that the overall polarity of the molecule did not change with the chain length of the analyte.

An interesting physical property of the methyl esters was that most of the compounds were liquid at room temperature. The only compounds that were solid at room temperature were methyl 9-thianonadecanoate and methyl 9-thiaeicosanoate. The addition of a sulfide bond into a hydrocarbon chain introduces a small bend in the molecule, due to the bond angle being *ca.* 96° as opposed to that of carbon, 109° .⁴⁸ The

bend that is present in the molecule inhibits the molecular stacking of the chains, thus decreasing the melting point of the compound.⁴⁸ This is evident in comparing methyl hexadecanoate, (palmitate), which has a melting point range of 32 °C - 34 °C, to methyl 9-thiapalmitate, a liquid at 14 °C.

Figure 2.2.2 Methyl 9-Thiapalmitate

The ^1H NMR spectra of all compounds are consistent with their respective assigned structures. The spectra contain the characteristic triplet for the C-2 group, (δ 2.28 ppm, 3J 7.5 Hz), as well as the singlet for the methyl hydrogens of the ester (δ 3.64 ppm).⁴⁹ The essential ^1H NMR spectral features were almost identical for the esters synthesized from methyl 9-thiatridecanoate to methyl 9-thiaeicosanoate. A detailed description follows with respect to the main compound of interest: methyl 9-thiapalmitate.

The ^1H NMR spectrum, (Figure 2.2.3), of methyl 9-thiapalmitate, contains the characteristic overlapping triplets, for C-8 and C-10, (δ 2.47 ppm, 3J 7.3 Hz), along with the characteristic triplet of C-16, (δ 0.85 ppm 3J 7.1 Hz). These coupling constants (5 – 10 Hz), as well as the chemical shifts are in agreement with literature values.⁴⁹

Figure 2.2.3 ^1H NMR Spectrum (200 MHz) of Methyl 9-Thiahexadecanoate

As anticipated, the ^1H NMR spectra of the methyl 9-thiadecanoate, methyl 9-thiaundecanoate, as well as methyl 9-thiadodecanoate all differed from that of methyl 9-palmitate in two areas. Firstly, the hydrogens adjacent to the sulfide give a different splitting pattern for each of the aforementioned compounds.⁵⁰ Secondly, the resonance for the triplet of the terminal methyl group shifts downfield the closer the carbon is to the sulfide.⁵⁰

The ^{13}C NMR spectra of all compounds are also in agreement with their respective assigned structures. The spectra contain signals corresponding to C-1 (δ 174.53 ppm), and the methyl carbon of the ester (δ 51.73 ppm). All of the key peaks of interest are similar for each of the chain lengths from methyl 9-thiatridecanoate to methyl 9-thiaeicosanoate. Further details will now be discussed for the main compound of interest: methyl 9-thiapalmitate.

Figure 2.2.4 ^{13}C NMR Spectrum (50.3 MHz) of Methyl 9-Thiapalmitate

The ^{13}C NMR spectrum of methyl 9-thiapalmitate, (Figure 2.2.4), exhibits the signals for the carbons on either side of the sulfur, C-10 and C-8, (δ 32.43 ppm, δ 32.35 ppm). Also visible is the signal for C-16, (δ 14.36 ppm). The peak assignment is in agreement with the literature values.⁴⁹

The ^{13}C NMR spectra of the methyl 9-thiadecanoate, methyl 9-thiaundecanoate, as well as methyl 9-thiadodecanoate all differ from that of methyl 9-thiapalmitate in two areas. Firstly, the signal for the eighth carbon of methyl 9-thiadecanoate differs in the chemical shift compared to that of the C_{16} analogue, due to its close proximity to the end of the chain. This effect also causes the position of the signal for the tenth carbon to fluctuate for all three compounds. Secondly, the resonance for the terminal methyl group shifts downfield, similar to the ^1H NMR, as the chain length decreases, due to the deshielding effects of the sulfide.⁴⁹

The GC/MS analysis of the methyl esters confirms purity, molecular weight, and position of the sulfur atom. The gas chromatogram, (Figure 2.2.5), showed a single peak, which is indicative of its high purity, > 99 %, and the mass spectrum, (Figure 2.2.6), confirmed the molecular weight, as well as the characteristic fragmentation pattern for the given sulfide, (C_{10} to C_{20}).

Figure 2.2.5 Gas Chromatogram of Methyl 9-Thiopalmitate

Figure 2.2.6 Mass Spectrum of Methyl 9-Thiopalmitate

The mass spectrum contains the molecular ion peak, $[m/z\ 288]$, as well as the ion due to the cleavage of the methyl ester, $[m/z\ 257, (M - OCH_3)^+]$. Two other fragments of interest $[m/z\ 157 ((CH_2)_7COOCH_3)^+]$, and $[m/z\ 189 (S(CH_2)_7COOCH_3)^+]$, are due to cleavage of the chain on either side of the sulfur atom, demonstrating that it is located in the ninth position.

2.2.2 Racemic Sulfoxides

The previously described sulfides were oxidized by the reaction of an equimolar amount of the mild oxidizing agent, *m*-chloroperoxybenzoic acid (Figure 2.2.7). The resulting methyl sulfoxide was purified by flash chromatography (silica gel, 100 % EtOAc). These compounds were used as reference standards for the biological chain length study.

Figure 2.2.7 Synthesis of Methyl 9-Sulfoxy Fatty Acids

Analysis of the TLC data obtained for the compounds, showed that their mobility on silica gel was greatly reduced relative to the corresponding sulfide. In addition, the R_f values, (100 % EtOAc), increased with an extension of the analyte chain length. This makes sense as an increase of the non-polar contribution is caused by the lengthening of the alkyl chain. It can be seen that the difference in R_f values is greatest for the series, C_{10} , ($R_f\ 0.09$), C_{11} , ($R_f\ 0.13$), C_{12} , ($R_f\ 0.21$), C_{13} , ($R_f\ 0.25$), and C_{14} , ($R_f\ 0.29$). This trend

becomes less pronounced at higher chain lengths of sulfoxide; C₁₅, C₁₆ (R_f 0.32), C₁₇, C₁₈ (R_f 0.36), C₁₉, C₂₀ (R_f 0.38).

The sulfoxides were characterized using ¹H NMR, as well as ¹³C NMR. GC/MS could not be applied to confirm the structure due to the internal elimination of the sulfoxide upon pyrolysis.⁵¹ The ¹H NMR spectra, of the methyl 9-sulfoxy fatty acids, all featured the singlet of the methyl ester hydrogens (δ 3.60 ppm), as well as the triplet for the C-2 hydrogens, (δ 2.24 ppm, ³J 7.5 Hz). The ¹H NMR spectra for the esters of chain lengths between thirteen and twenty carbons were very similar. Characterization of the sulfoxy-fatty acid of interest, methyl 9-sulfoxypalmitate, will be discussed.

Figure 2.2.8 Methyl 9-Sulfoxypalmitate

Figure 2.2.9 ¹H NMR Spectrum (400 MHz) of Methyl 9-Sulfoxypalmitate

The ^1H NMR spectrum, (Figure 2.2.9), contains the distinctive triplet for C-16, (δ 0.82 ppm, 3J 6.8 Hz), as well as a large multiplet, (δ 2.58 ppm), that is indicative of the diastereotopic hydrogens on C-8 and C-10. The complexity of this peak is due to the combination of geminal, (2J), and vicinal, (3J), coupling to neighbouring hydrogens, thus giving a doublet of doublet of doublets splitting pattern, (Figure 2.2.10). In addition, the signals for the hydrogens at C-8 and C-10 appear at slightly different chemical shift values and overlap.

Figure 2.2.10 Geminal and Vicinal Coupling of C-8 Hydrogens

The ^1H spectrum also features a complicated multiplet at δ 1.76 ppm, which is due to the four hydrogens β to the sulfoxide.⁴⁹

The spectrum of methyl 9-sulfoxypalmitate contrasts from those of methyl 9-sulfoxidecanoate, methyl 9-sulfoxundecanoate, and methyl 9-sulfoxidodecanoate in the chemical shifts and coupling of the hydrogens adjacent to the sulfoxide, (Figure 2.2.11), as well as in the terminal methyl group.

Figure 2.2.11 Chemical Shifts in the ^1H NMR for C-8 and C-10 in
(A) Methyl 9-Sulfoxypalmitate
(B) Methyl 9-Sulfoxidecanoate
(C) Methyl 9-Sulfoxundecanoate
(D) Methyl 9-Sulfoxydodecanoate

The assignment of the ^{13}C NMR spectra for the methyl sulfoxides was based on a similar analysis previously carried out for methyl 9-sulfoxystearate.⁴³ All spectra featured resonances due to C-1, (δ 174.4 ppm), as well as the methyl ester carbon, (δ 51.7 ppm). The ^{13}C NMR spectrum of methyl 9-sulfoxypalmitate will be discussed in detail to further characterize the structure.

Figure 2.2.12 ^{13}C NMR Spectrum (100.6 MHz) of Methyl 9-Sulfoxypalmitate

The ^{13}C NMR spectrum of methyl 9-sulfoxypalmitate, (Figure 2.2.12), contains the characteristic signal for C-16, (δ 13.98 ppm), as well as the resonances for C-10, (δ 52.78 ppm), and C-8, (δ 52.65 ppm). These were assigned by comparison with spectra obtained previously for 10- d_2 -methyl 9-sulfoxystearate.⁴⁶

Table 2.2.1 Table of Diagnostic Resonances of Methyl 9-Sulfoxy Fatty Acids

Compound	ω C	C ₁₀	C ₈	C ₇	C ₂	C ₁
Decanoate	-	38.80	54.92	22.75	34.22	174.42
Undecanoate	7.05	45.89	51.88	22.79	34.23	174.43
Dodecanoate	13.69	54.65	52.65	22.79	34.21	174.42
Tridecanoate*	13.96	52.63	52.46	22.81	34.23	174.44
Tetradecanoate	14.10	52.68	52.59	22.80	34.23	174.44
Pentadecanoate	14.23	52.72	52.59	22.80	34.22	174.43
Hexadecanoate*	13.28	52.77	52.64	22.90	34.23	174.40
Heptadecanoate	14.34	52.73	52.60	22.87	34.23	174.43
Octadecanoate	14.37	52.74	52.60	22.81	34.23	174.45
Nonadecanoate	14.37	52.75	52.61	22.81	34.23	174.42
Eicosanoate	14.39	52.75	52.62	22.82	34.24	174.21

*these values were normalized relative to the average C₂ value of 34.23

Table 2.2.1 contains the characteristic ^{13}C NMR peaks for the compounds. The greatest change of the carbon signals occur in the sulfoxy region for the compounds with a chain length less than C_{14} . The underlying principle for the variance of the signals was previously discussed, (Section 2.2.1). As is clearly shown in Table 2.2.1, there is a negligible difference in the chemical shifts for the eighth and tenth carbon of the C_{16} and C_{18} chain length, thus, allowing a comparison of the two spectra.

2.2.3 Formation of Methyl 9-Sulfonylpalmitate

The oxidation of methyl 9-thiapalmitate with two and a half molar equivalents of *meta*-chloroperoxybenzoic acid produced the title compound, (Figure 2.2.13). The resulting methyl 9-sulfonylpalmitate was purified by flash chromatography (silica gel, 2.5 % EtOAc/Hex) to give a colourless solid (92 % yield).

Figure 2.2.13 Synthesis of Methyl 9-Sulfonylpalmitate

Initial TLC analysis of the title compound indicates that the compound was indeed pure. An interesting physical property of the sulfone group, is denoted via analysis of the TLC data. The R_f values for methyl 9-sulfoxypalmitate, (0.32, 100 % EtOAc), and methyl 9-sulfonylpalmitate (0.92, 100 % EtOAc), indicates that the sulfone is far less polar than the sulfoxide, whereas the R_f values of methyl 9-sulfonylpalmitate, (0, 2.5 %, EtOAc/Hex), and methyl 9-thiapalmitate, (0.13, 2.5 % EtOAc/Hex), show that the sulfone has a higher polarity than the sulfide. In the analysis of the net dipoles for

dimethyl sulfone, (4.49 D), and dimethyl sulfoxide, (3.96 D), it can be seen that the sulfone has a larger dipole moment.²⁴ However, the analysis of the TLC results show that the sulfoxide group is more effective at hydrogen bonding than the corresponding sulfonyl group. This difference may be credited to a weaker individual dipole moment for each sulfur-oxygen bond in the sulfone.³²

Figure 2.2.14 Methyl 9-Sulfonylpalmitate

The ^1H NMR spectrum methyl 9-sulfonylpalmitate, (Figure 2.2.15), is similar to that of the racemic methyl 9-sulfoxypalmitate previously discussed, (Section 2.2.2). The primary difference is the chemical shift of the signal representing the C-8 and C-10 hydrogens, (δ 2.94 ppm, 3J 8.1 Hz). The doubling of the signals is due to the overlapping chemical shifts of the C-8 and C-10 hydrogens.

Figure 2.2.15 ^1H NMR Spectrum (400 MHz) of Methyl 9-Sulfonylpalmitate

The ^{13}C NMR spectrum, (Figure 2.2.16), displayed the expected peaks that correspond to methyl 9-sulfonylpalmitate. In particular, the signal due to the eighth carbon, (δ 52.65 ppm), as well as the tenth carbon, (δ 52.81 ppm), lie in the expected region for carbons adjacent to a sulfonyl functional group.⁴⁹

Figure 2.2.16 ^{13}C NMR Spectrum (100.6 MHz) of Methyl 9-Sulfonylpalmitate

2.3 Biological Experiments

2.3.1 Chain Length Study

The main focus of this experiment was to determine which chain length of thia fatty acid would give the greatest amount of an enantiomerically enriched 9-sulfoxide upon incubation with *Saccharomyces cerevisiae*. High efficiency is needed for the process to produce the *ca.* 100 milligram quantities that are needed in the synthesis of phospholipids. *S. cerevisiae* incorporates the thia fatty acid methyl ester and attempts Δ^9 desaturation of the substrate as the CoA ester. Due to the initial site of oxidative attack of the Δ^9 desaturase at the pro-*R* C-9 position, the yeast cell can be used to produce (*R*)-chiral sulfoxides.^{46,52,53} Upon formation of the chiral sulfoxide, the cell excretes this

compound into the supernatant as the free fatty acid. Extraction of the fatty acid with chloroform was performed after careful acidification of the supernatant to *ca.* pH 3 with glacial acetic acid. Analysis and purification of the crude sulfoxide needed to be completed using methyl ester forms of the free acids. Methylation of the supernatant could not occur via Fischer esterification, due to the possibility of racemization of the sulfoxide. It has been found that even weak acids are effective racemizing agents when a solution is heated.³² Since methylation using BF_3/MeOH also requires refluxing, another route had to be taken. One possible synthetic route for methylation that requires mild and neutral conditions is the use of diazomethane.⁵⁴

Figure 2.3.1 Synthesis of Nitrosomethyl Urea

Nitrosomethyl urea was formed by refluxing concentrated hydrochloric acid with methylamine and urea. The resulting solution was mixed with sodium nitrite and warmed, yielding a homogeneous solution. The desired compound was precipitated out of solution as a yellow solid, upon acidification with concentrated sulfuric acid. The compound was kept at 0 °C until needed. The conversion of nitrosomethyl urea into diazomethane requires a basic solution. The nitrosomethyl urea was mixed with a two molar potassium hydroxide solution, initiating hydrolysis, and causing the release of nitrogen gas and diazomethane, giving the ethereal phase a yellow colour. The ethereal phase was then ready to use as a methylating agent for the supernatant extracts.

Figure 2.3.2 Methylation of Fatty Acid via Diazomethane

The most appropriate method of screening the supernatant fractions for sulfoxide content remained to be determined. The supernatant of the control run, which involved the growth of the yeast under the same conditions as the sulfide incubations, in the absence of the substrate, was analyzed. Analysis of the ^1H NMR spectrum, (Figure 2.3.3), for the control sample, showed that there are no peaks present in the area where the hydrogens adjacent to the sulfide resonate, and only a small cluster of signals in the range characteristic of the hydrogens next to a sulfoxide. This result determined that the ^1H NMR spectrum was a plausible measuring tool for the presence of sulfide and sulfoxide in the supernatant extract.

Figure 2.3.3 ^1H NMR Spectrum (400 MHz) of the Supernatant, (Control Sample)

Another stumbling block was the question of how to quantitate the sulfide and sulfoxide present in the supernatant extract. To accomplish this, an internal standard was added to the sample, making it possible to compare signal integration. The criterion for the standard to be used was that it contained a proton signal, in an area that was not already characteristic of the control sample, sulfide or sulfoxide product. The compound that was selected was methyl *S*-(+)- α -methoxyphenylethanoate.

Figure 2.3.4 Synthetic Route of Methyl *S*-(+)- α -Methoxyphenylethanoate

Methyl *S*-(+)- α -methoxyphenylethanoate, (Figure 2.3.4), was formed by the reaction of *S*-(+)- α -methoxyphenylethanoic acid and methanol in the presence of boron trifluoride diethyletherate. The structure was confirmed using the ^1H NMR spectrum, (Figure 2.3.5). The spectrum contained the singlets that were representative of the methyl groups of the ether, (δ 3.39 ppm), and the ester, (δ 3.70 ppm), as well as a multiplet signalling the phenyl hydrogens, (δ 7.38 ppm). The most important peak was a singlet that is representative of the chiral hydrogen, (δ 4.76 ppm), which does not interfere with any of the peaks that are present in the spectra of the control experiment, sulfides or sulfoxides.

Figure 2.3.5 ^1H NMR Spectrum (200 MHz) of Methyl *S*-(+)- α -methoxyphenylethanoate

Having resolved all of the analytical issues, incubation of the sulfides, ($\text{C}_{10} - \text{C}_{20}$), with growing cultures of yeast, (20 mg/mL, 250 mL total volume), was completed over

24 hours, running 3 trials at a time within 30 minutes of each other. This approach was taken to ensure that all of the trials were completed under the same conditions.

Figure 2.3.6 Chain Length Study

For the calculation of the amount of sulfide and sulfoxide recovered from the supernatant, a known amount of the standard was added to the ^1H NMR sample. The percent recovered values were obtained by comparing the integration of the singlet of the reference standard, (δ 4.76 ppm), to that of the signals representing the hydrogens on C-8 and C-10, for the sulfide, (δ 2.49 ppm), and the sulfoxide, (δ 2.66 ppm). The sulfoxide integration was corrected to account for the overlapping peaks present in the control sample. Through analysis of the results, (Figure 2.3.6), it can be seen that there is an

optimum chain length accepted into the yeast, C₁₅ and C₁₆. The results also show that the C₁₅ chain length produced the largest amount of sulfoxide, which correlates with previous work in the Buist lab.⁵⁵ Studies of chain length dependence on the desaturation of 5-thia fatty acids, (C₁₄ to C₂₀), by *S. cerevisiae*, revealed that there was a similar chain length dependence on desaturation.⁵⁵

Figure 2.3.7 Desaturation of 5-Thia Fatty Acids

2.3.2 Chiral Methyl 9-Sulfoxypalmitate

The large scale production of the enantiomerically enriched methyl 9-sulfoxypalmitate was completed on the same scale, using the previously discussed method, (Section 2.3.1). The work-up of the supernatant gave an impure extract as a brown solid. Methyl 9-sulfoxypalmitate was purified via flash chromatography (100 % EtOAc), yielding 10 mg of a yellowish solid per run. This method was repeated to attain the required amount for the phospholipid synthesis, (200 mg).

The ¹H NMR spectrum, (Figure 2.3.8), and the ¹³C NMR spectrum, (Figure 2.3.9) of the biosynthetic methyl 9-sulfoxypalmitate was in agreement with assigned structure, and quite similar to that of the racemic standard that was synthesized. The spectra displayed the characteristic peaks as previously discussed, (Section 2.2.2).

Figure 2.3.8 ^1H NMR Spectrum (400 MHz) of Biosynthetic Methyl 9-Sulfoxypalmitate

Figure 2.3.9 ^{13}C NMR Spectrum (100.6 MHz) of Biosynthetic Methyl 9-Sulfoxypalmitate

2.3.3 Stereochemistry of Methyl 9-Sulfoxypalmitate Produced via Bio-sulfoxidation

The absolute configuration and enantiomeric excess of the title compound was determined by the use of the chiral shift reagent, *S*-(+)- α -methoxyphenylacetic acid. To set up this method, the NMR sample of racemic methyl 9-sulfoxypalmitate was mixed with three molar equivalents of the chiral shift reagent. The shift reagent is able to hydrogen bond to the oxygen as well as to the lone pair on the sulfur of the sulfoxide functional group, (Figure 2.3.10).

Figure 2.3.10 Complexation of Methyl 9-Sulfoxypalmitate with *S*-(+)- α -Methoxyphenylacetic Acid

The hydrogen bonding of the shift reagent to the sulfoxide will have an effect on its neighbouring hydrogens. Deuterium labelling studies have revealed the deshielding effects of the hydrogen bonding induces the splitting of the resonances for H_A and H_B , (Figure 2.3.11).⁴³ The complexity of the signals prevented further analysis of the stereochemistry.

Figure 2.3.11 ^1H NMR Spectrum (400 MHz) of Racemic Methyl 9-Sulfoxypalmitate in the Presence of Three Molar Equivalents of *S*-(+)- α -Methoxyphenylethanoic Acid

The signals for C-8, (δ 52.65 ppm), and C-10, (δ 52.78 ppm), in the ^{13}C NMR spectrum of the racemic methyl 9-sulfoxypalmitate appear as singlets. The addition of the shift reagent causes an upfield shift of these peaks, due to the effect of hydrogen bonding, (Figure 2.3.12).

Figure 2.3.12 Complexing of Methyl 9-Sulfoxypalmitate with *S*-(+)- α -Methoxyphenylacetic Acid

The secondary through space shielding effect of the phenyl ring causes a doubling of the signals for the eighth and tenth carbon.⁴⁶ This effect is exhibited in the spectrum of the racemic mixture, (Figure 2.3.13), by the signals for C-10, [δ 51.21 ppm (*R* form), δ 51.17 ppm (*S* form)], and C-8, [δ 51.08 ppm (*S* form), δ 51.05 ppm (*R* form)].

Figure 2.3.13 ^{13}C NMR Spectrum (100.6 MHz) of Racemic Methyl 9-Sulfoxypalmitate (A) Before (B) After Shift Reagent Addition

The mixture of the enantiomerically enriched substrate and the shift reagent should not show the doubling of the peaks, due to the compound only being present in the *R* form. This is evident in the ^{13}C NMR spectrum, (Figure 2.3.11), where only one singlet representing C-10, (δ 51.41 ppm), as well as one singlet for C-8, (δ 51.26 ppm), are present.

Figure 2.3.14 ^{13}C NMR Spectrum (100.6 MHz) of Enantiomerically Enriched Methyl 9-Sulfoxypalmitate (A) Before (B) After Shift Reagent Addition

The presence of shoulders on the resonances for the enantiomerically enriched substrate, (Figure 2.3.14), can allow us to make a conservative estimate that the compound > 90 % enantiomerically “pure”. The presence of the shoulders on the resonances for C-8 and C-10 is in agreement with the previous studies on methyl 9-*R*-sulfoxystearate.⁴⁶ Mixing of the enantiomerically enriched and racemic compounds further proved that the biosynthetic methyl 9-sulfoxypalmitate was of the *R* configuration. The ^{13}C NMR spectrum for a 2:1 mixture of enantiomerically enriched and racemic 9-sulfoxypalmitate, (Figure 2.3.15), exhibited smaller resonances for C-8 and C-10 in the *S* configuration.

Figure 2.3.15 ^{13}C NMR Spectrum (100.6 MHz) of Enantiomerically Enriched and Racemic (2:1) 9-Sulfoxypalmitate

2.4 Phospholipid Synthesis

2.4.1 Saponification

Phospholipids are synthesized by condensing fatty acids with substituted glycerols. Hydrolysis of the purified thiapalmitate analogue methyl ester is needed to obtain the free fatty acid form. In this reaction, the given methyl ester is mixed with a two molar solution of potassium hydroxide in a 50 % ethanol/distilled water solvent. The methyl ester did not dissolve into the solution, thus heat was added to accelerate the reaction, forming the water soluble carboxylate anion. Once a homogeneous solution was present, the solution was allowed to cool to room temperature, and was acidified to pH 2 via 3M sulfuric acid, to precipitate the insoluble carboxylic acid. It was important to use dilute sulfuric acid to quench the anions present instead of hydrochloric acid due to the possible racemization effect the chlorine anion may have on the enantiomerically enriched sulfoxide.³²

The ^1H NMR and ^{13}C NMR spectra of 9-thiapalmitic acid, 9-sulfonylpalmitic acid, 9-(*R,S*)-sulfoxypalmitic acid, and 9-(*R*)-sulfoxypalmitic acid are very similar to their respective methyl esters. The absence of the methyl ester peak in the ^1H spectra, (*ca.* δ 3.65 ppm), and in the ^{13}C spectra, (*ca.* 51.73 ppm), indicates that the hydrolysis reaction was a success. Another feature of the ^{13}C NMR spectra was the downfield shift of C-1 (*ca.* δ 179.83 ppm).

An interesting splitting pattern for the C-8 and C-10 hydrogens is present in the ^1H NMR for the racemic and enantiomerically enriched 9-sulfoxypalmitic acid, (Figure 2.4.1). The doublet of multiplets is due to the internal hydrogen bonding of the sulfoxide to the proton on the carboxylic acid functional group, as previously described, (Section 2.3.3).

Figure 2.4.1 ^1H NMR Spectrum (400 MHz) for 9-(*R,S*)-Sulfoxypalmitic Acid

2.4.2 Synthesis of Phospholipids

Phospholipid synthesis was carried out by reacting L- α -glycerophosphorylcholine with the appropriate fatty acid, in the presence of dicyclocarbodiimide, (DCC), and dimethyl-4-aminopyridine, (DMAP), to give the desired compound in yields ranging from 25 % to 46 %, (Figure 2.4.2).

Figure 2.4.2 Synthesis of Phospholipids

Care was taken to ensure the reaction was carried out under anhydrous conditions, and in the absence of any alcohol functional group that may prevent the reaction from going to

completion. The reaction was completed in chloroform, which is usually stabilized by the addition of up to 1 % ethanol or dimethylaminobenzene.⁵⁶ Previously published methods called for distillation of the solvent over phosphorus pentoxide to remove the ethanol.¹⁸ However, the solvent for the reaction was changed to deuterated chloroform, which does not contain ethanol. The L- α -glycerophosphorylcholine was dried in *vacuo*, using a drying pistol submersed in an acetone reflux, (56 °C). The fatty acids that were used were dehydrated via repeated evaporation of acetonitrile, added as an azeotroping agent, and concentrated to dryness using a soft, steady flow of nitrogen. The coupling reaction was completed under a slow flowing blanket of nitrogen, in a flame dried reaction vessel.

The presence of DMAP and DCC facilitated the addition of the fatty acid tail group to the head group, phosphatidylcholine, in the formation of the phospholipid. DMAP was used as a proton acceptor, to initiate the reaction between the hydroxyl group of the fatty acid and DCC. DCC acted as an agent to remove the hydroxyl group of the carboxylic acid as well as the hydrogen of the alcohols attached to the phosphatidylcholine group, to form *N,N*-dicyclohexylurea.

Figure 2.4.3 Dipalmitoyl-3-*sn*-Phosphatidylcholine (DPPC)

The ^1H NMR spectra of the phospholipids were identical in the peak pattern representing the headgroup. A singlet represents the C-1 hydrogens, (δ 3.38 ppm), broad signals are present for C-2, (δ 3.82 ppm), and the C-3 hydrogens, (δ 4.32 ppm).⁵⁷ Two overlapping doublet of doublets are present due to the C-4 hydrogens, (δ 3.95 ppm, 2J 11.0 Hz, 3J 7.3 Hz).⁵⁷ The hydrogens for C-6 is responsible for the two doublet of doublets at higher field, (δ 4.13 ppm, 2J 12.0 Hz, 3J 7.3 Hz; δ 4.40 ppm, 2J 12.0 Hz, 3J 2.8 Hz).⁵⁷ The difference in the vicinal coupling of the hydrogens is due to their dihedral angle. There are two possible orientations for the hydrogens: *gauche* and *trans*. The *gauche* conformation generally displays a coupling constant of *ca.* 2 – 4 Hz, while the coupling of the *trans* conformer typically ranges from 8 – 12 Hz.⁵⁰ Finally the multiplet at δ 5.20 ppm can be assigned to the C-5 hydrogen.⁵⁷

Figure 2.4.4 ^1H NMR Spectrum (400 MHz) of DPPC

The ^1H spectrum for the fatty acid tail of DPPC featured a triplet for the C-16'/C16'' hydrogens, (δ 0.88 ppm, 3J 6.8 Hz), and a broad peak for the hydrogens on C-3'/C-3'', (δ 1.57 ppm). The presence of a doublet of triplets, (δ 2.28 ppm, 3J 7.6 Hz), is due to the proximity of C-2' to the chiral centre of the glycerol group overlapping with the C-2'' signal. This attribute indicates that the coupling reaction was successful. The ^1H NMR spectra for the other phospholipids differed from that of DPPC in the tail group only. The characteristic signals of the tail group were similar to that of their respective fatty acid methyl esters, which were previously discussed, DP(S)PC, (Section 2.2.1), rDP(SO)PC, (Section 2.2.2), cDP(SO)PC, (Section 2.3.2), and DP(SO₂)PC, (Section 2.4.1).

The ^{13}C NMR spectra of the phospholipids were also identical in the signals for the phospholipid head group. The signal for C-1 as a singlet, (δ 54.49 ppm).⁵⁷ The resonance for C-3, (δ 59.30 ppm, 3J 4.8 Hz), appears as a doublet due to the $^{13}\text{C} - ^{31}\text{P}$ coupling, which is agreement with the literature value *ca.* 7 Hz.⁴⁹ The resonance for C-6, (δ 63.03 ppm), appears as a singlet, because it is out of the coupling range of the phosphorus.⁴⁹ Signals for C-4, (δ 63.38 ppm, 2J 5.0 Hz), C-2, (δ 66.44 ppm, 3J 5.8 Hz) and C-5, (δ 70.58 ppm, 3J 7.5 Hz) also appeared as doublets.⁵⁷

Figure 2.4.5 ^{13}C NMR Spectrum (100.6 MHz) of DPPC

The ^{13}C NMR spectrum of DPPC, (Figure 2.4.5) contained the characteristic resonances of the fatty acid tail group including those for C-16'/C-16'', (δ 14.14 ppm), C-2'/C-2'', (δ 34.16 ppm, δ 34.37 ppm), and C-1'/C-1'', (δ 173.24 ppm, δ 173.59 ppm). The presence of two signals for the groups close to the chiral centre of the glycerol, again, indicates that the coupling reaction was a success. The ^{13}C NMR spectra for the tail group region of the other phospholipids varied as expected from that of DPPC. The distinctive signals of the tail group were similar to that of their respective fatty acids, which were previously discussed, DP(S)PC, (Section 2.2.1), rDP(SO)PC, (Section 2.2.2), cDP(SO)PC, (Section 2.3.2), and DP(SO₂)PC, (Section 2.4.1).

The molecular weights of the phospholipids were confirmed via the use of electrospray mass spectrometry analysis. All phospholipids exhibited their respective molecular ion peak as well as the peak for the sodium salt in their spectra. The mass

spectrum of DPPC, (Figure 2.4.6), contained the expected molecular ion peak, (m/z 734), as well as a peak for the sodium salt. Analysis of the phospholipids was completed for the cations. When the analysis of the anion was attempted, the molecular ion peak was not found, due to the presence of the sodium cation.

Figure 2.4.6 Mass Spectrum of DPPC

2.5 Phospholipid Studies

2.5.1 Stereochemistry of 9',9''-Disulfoxypalmitoyl-3-*sn*-phosphatidylcholine

In order to confirm that racemization did not occur in the coupling reaction, the phospholipids, bearing the sulfoxide, were tested for enantiomeric excess using *S*-(+)- α -methoxyphenylacetic acid as the chiral shift reagent. This method differed from that previously discussed, (Section 2.3.2), by the addition of eight molar equivalents of shift reagent.

Figure 2.5.1 Comparison of the Signals for C-8', C-8'' and C-10', C10'' of rDP(SO)PC (A) Before (B) After Shift Reagent Addition

Figure 2.5.2 – Comparison of the Signals for C-8', C-8'' and C-10', C-10'' of cDP(SO)PC (A) Before (B) After Shift Reagent Addition

As previously discussed, (Section 2.3.3), the racemic mixture exhibited a shifting as well a doubling of the signals representing the carbons adjacent to the sulfoxide, whereas the enantiomerically enriched compound displayed no such splitting. The racemic sample, (Figure 2.5.1), differs from the previous study in that only one carbon signal is showing the splitting, whereas, the enantiomerically enriched sample, (Figure 2.5.2), shows the appearance of a shoulder on the same carbon. The presence of the

shoulder may indicate a small degree of racemization. However, the use of a higher field instrument could verify or disprove this hypothesis.

2.5.2 Preliminary Langmuir Film Studies

Langmuir film balance experiments were completed on the enantiomerically enriched and racemic phospholipids, at varying temperatures, to determine if the presence of the enantiomerically enriched sulfoxide moiety resulted in improved dipole stacking over its racemic analogue. The pressure/area isotherms, (Figure 2.5.3), shows that the onset of the liquid phase occurs at a smaller area/molecule for the enantiomerically enriched sample. This suggests an improvement in packing due to dipole-dipole alignment of the chiral moieties. This difference in packing is further proof that racemization did not occur during phospholipid synthesis.

Figure 2.5.3 Pressure/Area Isotherm of cDP(SO)PC and rDP(SO)PC at 21°C

2.6 Future Studies

Initial studies show promise of increased stability in the chiral phospholipids. The next step in solving the overall picture is to determine the effect of dipole-dipole alignment on the phase transition of the compound, via differential scanning calorimetry. Once the phase transition temperature is determined, stacking properties of the sulfoxide analytes can be compared to those of DPPC, DP(S)PC, and DP(SO₂)PC.

Chapter 3 Experimental

3.1 Materials and Methods

^1H NMR (400 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded on a Bruker AMX 400 spectrometer. ^1H NMR (200 MHz) and ^{13}C NMR (50.3 MHz) spectra were recorded on a Gemini 200 spectrometer. All compounds for NMR were dissolved in CDCl_3 that had been passed through neutral alumina. Chemical shifts for ^1H and ^{13}C NMR spectra are reported in ppm (δ) relative to tetramethylsilane (TMS) and CHCl_3 impurity in the deuterated solvent. Abbreviations used in assigning peaks are as follows: s = singlet, d = doublet, t = triplet, m = multiplet, b = broad, o = overlapping.

GC/MS data were collected at the University of Ottawa using a Kratos Concept 1H mass spectrometer at 70 eV interfaced with a J. & W. 30 m $\times$ 0.21 mm, DB-5 capillary column which was temperature programmed for 120 $^\circ\text{C}$, (held, 2 min) to 320 $^\circ\text{C}$ at 10 $^\circ\text{C}/\text{min}$. MS spectra were obtained using a direct probe. Electrospray data was obtained using a Micromass Quatropole LC using Mass Lynx software.

Autoclaving was carried out using a AMSCO 2021 gravity autoclave. All materials were sterilized using the liquid setting for 20 min with a pressure of *ca.* 1.2 kg per cm^2 . The cell cultures were incubated in a Model G25 Incubator Shaker, running at 150 RPM, at 30 $^\circ\text{C}$.

Purification by flash chromatography used Merck silica gel 60 (230-400 mesh) with an air pressure of *ca.* 27 kPa for phospholipids, and *ca.* 40 kPa for all of the other synthesized compounds. Thin layer chromatography was performed using Merck-coated silica gel (60 $\text{\AA}$) mounted on glass plates as well as Sigma-Aldrich-coated silica gel (60

Å) on aluminium plates, and developed using 5 % phosphomolybdic acid in ethanol, I₂ vapour chamber or water spray.

All reagents and starting materials for the syntheses were purchased from the Sigma-Aldrich Chemical group, and used without purification. All solvents used in reactions were purchased from Caledon Chemical Company and were of spectral grade quality. Tetrahydrofuran (THF) was distilled over sodium metal. With the exception of the oxidation process, all reactions were performed under nitrogen gas. Solvents were removed in *vacuo* using a Buchi RE 111 Rotovapour.

Reagents for the biological experiments were purchased from VWR canlab. Distilled deionized water, used in the biological experiments, was obtained from a Millipore Milli-Q water system. The Sorvall superspeed RC2-B automatic refrigerated centrifuge was used in the centrifugation of the biological experiments.

Melting points were determined using a Mel-Temp II capillary tube heating apparatus. The melting range was recorded using a Laboratory Devices Inc. digital thermometer (-160 – 500 °C).

Isotherms were recorded using a KSV 3000 Langmuir film-balance, (KSV Instruments), equipped with a 150 mm × 540 mm Teflon compartment. A circulating bath provided thermal control of the subphase. Solutions of *ca.* 1.5 mg/mL were made using HPLC grade CHCl₃. Samples were spread onto a subphase of deionized water via a µL syringe in dropwise fashion, and the solvent was allowed to evaporate over 15 minutes. A compression rate of 10 mm/min was used in each run.

3.2 Synthesis of Substrates

The route described below is for the synthesis of methyl 9-thiapalmitate. This is the same procedure used to synthesize all chain lengths from methyl 9-thiadecanoate to methyl 9-eicosanoate.

3.2.1 8-Thiooctanoic Acid

Absolute ethanol (150 mL) was added to a N₂ flushed round bottom flask that contained 8-Bromooctanoic acid (14.5082g, 0.065 mol). Thiourea (5.2085g, 0.068 mol) was added to the reaction vessel and the solution refluxed for 3 hours. All of the solids dissolved into solution, upon heating. The solution was allowed to cool to room temperature, and a freshly prepared solution of 2 M NaOH, in 1:1 EtOH:H₂O (240 mL) was added. A white precipitate formed upon the addition of the basic solution. The mixture was then refluxed for 1.5 hours, and became clear upon heating. The solution was cooled to room temperature over 1 hour, mixed with dH₂O (300 mL), and slowly introduced to a stirring solution of 6M H₂SO₄ (72 mL) and CH₂Cl₂ (160 mL). The phases were separated and the aqueous phase was extracted with CH₂Cl₂ (3 x 100 mL). The combined organic phases were backwashed with acidified (8 drops 6M H₂SO₄ in 100 mL) saturated NaCl solution (3 x 100 mL), dried over anhydrous Na₂SO₄, and

concentrated in *vacuo*. The compound was chased with CH_2Cl_2 and evaporated in *vacuo* to give 10.8694 g (0.062 mol, 94%) of the crude thiol product.

^1H NMR (400 MHz) δ 1.33 [b, 6H, $\text{R}-(\text{CH}_2)_3(\text{CH}_2)_2\text{COOH}$], δ 1.62 [m, 4H, $\text{HSCH}_2\text{CH}_2(\text{CH}_2)_3\text{CH}_2\text{CH}_2\text{COOH}$], δ 2.35 [t, 3J 7.5 Hz, 2H, $\text{R}-\text{CH}_2\text{COOH}$], δ 2.51 [t, 3J 7.5 Hz, 2H, HSCH_2-R], δ 11.58 [b, 1H, $-\text{OH}$]; ^{13}C NMR (100 MHz) δ 24.57, 24.59 (C-8 or C-3), δ 28.14, δ 28.69, δ 28.91, δ 33.93 (C-2), δ 33.98 (C-7), δ 180.01 (C-1)

3.2.2 Methyl 9-Thiapalmitate

Hexamethylphosphoramide, (HMPA) (15 mL), was added into a N_2 flushed, cooled, (0°C , ice bath), 250 mL 3-neck round bottom flask, that contained 8-thiooctanoic acid, (2.8873 g, 16.4 mmol), via a glass syringe. The solution was stirred for five minutes, freshly distilled tetrahydrofuran, (THF) (55 mL), was introduced via a plastic syringe, and the solution was mixed for an additional 5 minutes. Approximately 3 equivalents of n -butyllithium, ($n\text{-BuLi}$), in hexanes, (30 mL, 1.6 M, 48 mmol), was added to the reaction vessel slowly via a plastic syringe. A white precipitate initially formed upon the addition of $n\text{-BuLi}$, the solution was slightly brown in colour after the total

addition. The milky white mixture stirred for 1 hour at 0 °C. 1-Bromoheptane, (2.9 mL, 18.46 mmol), was added via a plastic syringe, and the reaction mixture stirred for 24 hours at room temperature. dH_2O , (100 mL), and 3 M HCl, (140 mL), was added to the solution. The aqueous phase was extracted with hexanes, (3×80 mL), and CH_2Cl_2 , (2×20 mL). The CH_2Cl_2 washes were combined, and concentrated in *vacuo* to give a yellow coloured oil, that was extracted with hexanes, (2×10 mL). The combined hexane fractions were dried over anhydrous Na_2SO_4 , concentrated in *vacuo*, and evaporated to constant weight under N_2 to give 4.9946g of the crude 9-thiapalmitic acid.

Crude 9-thiapalmitic acid, (4.9946g, 28.3 mmol), was dissolved in absolute methanol, (100 mL), and transferred to a N_2 flushed 3-neck round bottom flask. The reaction vessel was flushed with N_2 gas, and boron trifluoride diethyl etherate, (20 mL), was added. The system was flushed with N_2 and refluxed for 2 hours. The yellow coloured solution was cooled to room temperature, and mixed with dH_2O , (150 mL). The solution was extracted with CH_2Cl_2 , (3×50 mL), and the organic phases were combined, washed with saturated NaCl solution, (2×50 mL), dried over anhydrous Na_2SO_4 , and concentrated in *vacuo* to give 4.6061 g of the crude oil methyl 9-thiapalmitate. Purification by flash chromatography, (2.5% EtOAc/Hex), yielded 1.1367 g, (3.94 mmol, 24 % yield), of a colourless liquid at room temperature.

The purification for methyl 9-thiadeconoate, (233.3 mg), was completed by vigorously mixing 3 M NaOH, (5 mL), in a separatory funnel. The aqueous solution was extracted with CHCl_3 , (3×10 mL). The combined organic phases were backwashed with saturated NaCl solution, (15 mL), dried over Na_2SO_4 and concentrated in *vacuo* to give a colourless solution at room temperature.

- a. Methyl 9-Thiadecanoate:** ^1H NMR (200 MHz) δ 1.30 [b, 6H, $\text{R}-(\text{CH}_2)_3(\text{CH}_2)_2\text{COOCH}_3$], δ 1.56 [m, 4H, $\text{H}_3\text{CSCH}_2\text{CH}_2(\text{CH}_2)_3\text{CH}_2\text{CH}_2\text{COOCH}_3$], δ 2.06 [s, 3H, $\text{H}_3\text{CS-R}$], δ 2.27 [t, ^3J 7.4 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.45 [t, ^3J 7.3 Hz, 2H, $\text{H}_3\text{CSCH}_2\text{-R}$], δ 3.63 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 15.78 (C-10), δ 25.11 (C-3), δ 28.84 (C-7), δ 29.11, δ 29.26, δ 29.32, δ 34.28 (C-2), δ 34.48 (C-8), δ 51.68 ($-\text{OCH}_3$), δ 174.67 (C-1); MS (EI 70 eV) m/z 204 (M^+), 189 [$(\text{M} - \text{CH}_3)^+$], 173 [$(\text{M} - \text{OCH}_3)^+$], 157 [$(\text{M} - \text{SCH}_3)$].
- b. Methyl 9-Thiaundecanoate:** $R_f = 0.14$ (2.5 % EtOAc/Hex); ^1H NMR (200 MHz) δ 1.22 [t, ^3J 7.4 Hz, 3H, $\text{CH}_3\text{-CH}_2\text{S-R}$], δ 1.30 [b, 6H, $\text{R}(\text{CH}_2)_3(\text{CH}_2)_2\text{COOCH}_3$], δ 1.55 [m, 4H, $\text{H}_3\text{CCH}_2\text{SCH}_2\text{CH}_2(\text{CH}_2)_3\text{CH}_2\text{CH}_2\text{COOCH}_3$], δ 2.27 [t, ^3J 7.4 Hz, 2H, $\text{R-CH}_2\text{COO-CH}_3$], δ 2.48 [m, 4H, $\text{CH}_3\text{CH}_2\text{SCH}_2\text{-R}$], δ 3.63 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 15.05 (C-11), δ 25.11 (C-3), δ 26.17 (C-10), δ 28.97 (C-7), δ 29.12, δ 29.26, δ 29.80, δ 31.86 (C-8), δ 34.29 (C-2), δ 51.69 ($-\text{OCH}_3$), δ 174.48 (C-1); MS (EI 70 eV) m/z 218 (M^+), 189 [$(\text{M} - \text{CH}_2\text{CH}_3)^+$], 187 [$(\text{M} - \text{OCH}_3)^+$], 157 [$(\text{M} - \text{SCH}_2\text{CH}_3)$].
- c. Methyl 9-Thiadodecanoate:** $R_f = 0.17$ (2.5 % EtOAc/Hex); ^1H NMR (200 MHz) δ 0.97 [t, ^3J 7.4 Hz, 3H, $\text{H}_3\text{CCH}_2\text{-R}$], δ 1.31 [b, 6H, $\text{R}-(\text{CH}_2)_3(\text{CH}_2)_2\text{COOCH}_3$], δ 1.57 [m, 6H, $\text{H}_3\text{CCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2(\text{CH}_2)_3\text{CH}_2\text{CH}_2\text{COOCH}_3$], δ 2.29 [t, ^3J 7.3 Hz, 2H, $\text{R-CH}_2\text{-COOCH}_3$], δ 2.47 [m, 4H, $\text{H}_3\text{CCH}_2\text{CH}_2\text{SCH}_2\text{-R}$], δ 3.65 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 13.55 (C-12), δ 23.04 (C-11), δ 24.89 (C-3), δ 28.74, δ 28.88, δ

29.03 (C-7), δ 29.03, δ 29.67, δ 32.09, δ 34.08, δ 34.27 (C-2), δ 51.46 (-OCH₃), δ 174.28 (C1); MS (EI 70 eV) m/z 232 (M^+), 201 [(M - OCH₃)⁺], 189 [(M - (CH₂)₂CH₃)⁺], 157 [(M - S(CH₂)₂CH₃)⁺].

d. Methyl 9-Thiatridecanoate: $R_f = 0.14$ (2.5 % EtOAc/Hex); ¹H NMR (200 MHz) δ 0.88 [t, ³J 7.1 Hz, 3H, H₃CCH₂-R], δ 1.29 [b, 8H, H₃CCH₂(CH₂)₂S(CH₂)₂(CH₂)₃(CH₂)₂COOCH₃], δ 1.54 [m, 6H, H₃CCH₂CH₂CH₂SCH₂CH₂(CH₂)₃CH₂CH₂COOCH₃], δ 2.27 [t, ³J 7.1 Hz, 2H, R-CH₂COOCH₃], δ 2.47 [t, ³J 7.3 Hz, 4H, H₃C(CH₂)₂CH₂SCH₂-R], δ 3.63 [s, 3H, -OCH₃]; ¹³C NMR (50.3 MHz) δ 13.94 (C-13), δ 22.29 (C-12), δ 25.12 (C-3), δ 28.96 (C-7), δ 29.12, δ 29.26, δ 29.88, δ 32.10, δ 32.36, δ 34.30 (C-2), δ 51.68 (-OCH₃), δ 174.47 (C1); MS (EI 70 eV) m/z 246 (M^+), 215 [(M - OCH₃)⁺], 189 [(M - (CH₂)₃CH₃)⁺], 157 [(M - S(CH₂)₃CH₃)⁺].

e. Methyl 9-Thiatetradecanoate: $R_f = 0.13$ (2.5 % EtOAc/Hex); ¹H NMR (200 MHz) δ 0.87 [t, ³J 6.9 Hz, 3H, H₃CCH₂-R], δ 1.30 [b, 10H, H₃C(CH₂)₂(CH₂)₂S(CH₂)₂(CH₂)₃(CH₂)₂COOCH₃], δ 1.57 [m, 6H, H₃C(CH₂)₂CH₂CH₂SCH₂CH₂(CH₂)₃CH₂CH₂COOCH₃], δ 2.27 [t, ³J 7.4 Hz, 2H, R-CH₂COOCH₃], δ 2.46 [t, ³J 7.3 Hz, 4H, H₃C(CH₂)₃CH₂SCH₂-R], δ 3.63 [s, 3H, -OCH₃]; ¹³C NMR (50.3 MHz) δ 14.24 (C-14), δ 22.57 (C-13), δ 25.13 (C-3), δ 28.97 (C-7), δ 29.13, δ 29.27, δ 29.67, δ 29.88, δ 31.39 (C-12), δ 32.38, δ 32.42, δ 34.30 (C-2), δ 51.70 (-OCH₃), δ 174.49 (C1); MS (EI 70 eV) m/z 260 (M^+), 229 [(M - OCH₃)⁺], 189 [(M - (CH₂)₄CH₃)⁺], 157 [(M - S(CH₂)₄CH₃)⁺].

- f. Methyl 9-Thiapentadecanoate:** $R_f = 0.14$ (2.5 % EtOAc/Hex); ^1H NMR (200 MHz) δ 0.86 [t, 3J 6.5 Hz, 3H, $\text{H}_3\text{CCH}_2\text{-R}$], δ 1.29 [b, 12H, $\text{H}_3\text{C}(\text{CH}_2)_3(\text{CH}_2)_2\text{S}(\text{CH}_2)_2\text{-(CH}_2)_3\text{-(CH}_2)_2\text{COOCH}_3$], δ 1.55 [m, 6H, $\text{H}_3\text{C}(\text{CH}_2)_3\text{CH}_2\text{CH}_2\text{SCH}_2\text{CH}_2(\text{CH}_2)_3\text{-CH}_2\text{CH}_2\text{-COOCH}_3$], δ 2.27 [t, 3J 7.4 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.47 [t, 3J 7.3 Hz, 4H, $\text{H}_3\text{C}(\text{CH}_2)_4\text{CH}_2\text{SCH}_2\text{-R}$], δ 3.64 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 14.31 (C-15), δ 22.82 (C-14), δ 25.12 (C-3), δ 28.90, δ 28.97 (C-7), δ 29.13, δ 29.26, δ 29.86, δ 29.31, δ 31.72 (C-13), δ 32.34, δ 32.42, δ 34.29 (C-2), δ 51.71 ($-\text{OCH}_3$), δ 174.50 (C1); MS (EI 70 eV) m/z 274 (M^+), 243 [$(\text{M} - \text{OCH}_3)^+$], 189 [$(\text{M} - (\text{CH}_2)_5\text{CH}_3)^+$], 157 [$(\text{M} - \text{S}(\text{CH}_2)_5\text{CH}_3)^+$], 103 [$(\text{M} - (\text{CH}_2)_7\text{COOCH}_3)^+$].
- g. Methyl 9-Hexadecanoate:** $R_f = 0.13$ (2.5 % EtOAc/Hex); ^1H NMR (200 MHz) δ 0.86 [t, 3J 7.0 Hz, 3H, $\text{H}_3\text{CCH}_2\text{-R}$], δ 1.28 [b, 14H, $\text{H}_3\text{C}(\text{CH}_2)_4(\text{CH}_2)_2\text{S}(\text{CH}_2)_2(\text{CH}_2)_3\text{-(CH}_2)_2\text{COOCH}_3$], δ 1.56 [m, 6H, $\text{H}_3\text{C}(\text{CH}_2)_4\text{CH}_2\text{CH}_2\text{SCH}_2\text{CH}_2(\text{CH}_2)_3\text{CH}_2\text{CH}_2\text{-COOCH}_3$], δ 2.28 [t, 3J 7.5 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.48 [t, 3J 7.3 Hz, 4H, $\text{H}_3\text{CCH}_2\text{CH}_2\text{SCH}_2\text{-R}$], δ 3.65 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 14.36 (C-16), δ 22.89 (C-15), δ 25.13 (C-3), δ 28.98 (C-7), δ 29.15, δ 29.19, δ 29.28, δ 29.88, δ 29.98, δ 32.01 (C-14), δ 32.35, δ 32.43, δ 34.31 (C-2), δ 51.73 ($-\text{OCH}_3$), δ 174.53 (C1); MS (EI 70 eV) m/z 288 (M^+), 257 [$(\text{M} - \text{OCH}_3)^+$], 189 [$(\text{M} - (\text{CH}_2)_6\text{CH}_3)^+$], 157 [$(\text{M} - \text{S}(\text{CH}_2)_6\text{CH}_3)^+$], 117 [$(\text{M} - (\text{CH}_2)_7\text{COOCH}_3)^+$].
- h. Methyl 9-Thiaheptadecanoate:** $R_f = 0.14$ (2.5 % EtOAc/Hex); ^1H NMR (200 MHz) δ 0.88 [t, 3J 7.0 Hz, 3H, $\text{H}_3\text{CCH}_2\text{-R}$], δ 1.32 [b, 16H, $\text{H}_3\text{C}(\text{CH}_2)_5(\text{CH}_2)_2\text{S}(\text{CH}_2)_2\text{-(CH}_2)_3(\text{CH}_2)_2\text{COOCH}_3$], δ 1.57 [m, 6H, $\text{H}_3\text{C}(\text{CH}_2)_5\text{CH}_2\text{CH}_2\text{SCH}_2\text{CH}_2(\text{CH}_2)_3\text{-}$

$\text{CH}_2\text{CH}_2\text{COOCH}_3$], δ 2.30 [t, ^3J 7.5 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.49 [t, ^3J 7.4 Hz, 4H, $\text{H}_3\text{C}(\text{CH}_2)_6\text{CH}_2\text{SCH}_2\text{-R}$], δ 3.67 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 14.10 (C-17), δ 22.67 (C-16), δ 24.90 (C-3), δ 28.74, δ 28.90, δ 28.98 (C-7), δ 29.04, δ 29.22, δ 29.25, δ 29.66, δ 29.76, δ 31.84 (C-15), δ 32.15, δ 32.23, δ 34.07 (C-2), δ 51.46 ($-\text{OCH}_3$), δ 174.27 (C1); MS (EI 70 eV) m/z 302 (M^+), 271 [$(\text{M} - \text{OCH}_3)^+$], 189 [$(\text{M} - (\text{CH}_2)_7\text{CH}_3)^+$], 157 [$(\text{M} - \text{S}(\text{CH}_2)_7\text{CH}_3)$], 131 [$(\text{M} - (\text{CH}_2)_7\text{COOCH}_3)^+$].

- i. **Methyl 9-Thiaoctadecanoate:** $\text{R}_f = 0.16$ (2.5 % EtOAc/Hex); ^1H NMR (200 MHz) δ 0.85 [t, ^3J 6.8 Hz, 3H, $\text{H}_3\text{CCH}_2\text{-R}$], δ 1.26 [b, 18H, $\text{H}_3\text{C}(\text{CH}_2)_6(\text{CH}_2)_2\text{S}(\text{CH}_2)_2(\text{CH}_2)_3\text{-(CH}_2)_2\text{COOCH}_3$], δ 1.54 [m, 6H, $\text{H}_3\text{C}(\text{CH}_2)_6\text{CH}_2\text{CH}_2\text{SCH}_2\text{CH}_2(\text{CH}_2)_3\text{CH}_2\text{CH}_2\text{-COOCH}_3$], δ 2.27 [t, ^3J 7.4 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.46 [t, ^3J 7.2 Hz, 4H, $\text{H}_3\text{C}(\text{CH}_2)_7\text{CH}_2\text{SCH}_2\text{-R}$], δ 3.64 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 14.36 (C-18), δ 22.92 (C-17), δ 25.13 (C-3), δ 28.98 (C-7), δ 29.13, δ 29.22, δ 29.26, δ 29.53, δ 29.75, δ 29.88, δ 29.98, δ 32.13 (C-16), δ 32.37, δ 32.44, δ 34.30 (C-2), δ 51.69 ($-\text{OCH}_3$), δ 174.47 (C1); MS (EI 70 eV) m/z 316 (M^+), 285 [$(\text{M} - \text{OCH}_3)^+$], 189 [$(\text{M} - (\text{CH}_2)_8\text{CH}_3)^+$], 157 [$(\text{M} - \text{S}(\text{CH}_2)_8\text{CH}_3)$], 145 [$(\text{M} - (\text{CH}_2)_7\text{COOCH}_3)^+$].

- j. **Methyl 9-Thianonadecanoate:** $\text{R}_f = 0.15$ (2.5 % EtOAc/Hex); ^1H NMR (200 MHz) δ 0.85 [t, ^3J 6.5 Hz, 3H, $\text{H}_3\text{CCH}_2\text{-R}$], δ 1.24, 1.30 [b, 20H, $\text{H}_3\text{C}(\text{CH}_2)_7(\text{CH}_2)_2\text{S}(\text{CH}_2)_2\text{-(CH}_2)_3(\text{CH}_2)_2\text{COOCH}_3$], δ 1.55 [m, 6H, $\text{H}_3\text{C}(\text{CH}_2)_7\text{CH}_2\text{CH}_2\text{SCH}_2\text{CH}_2(\text{CH}_2)_3\text{CH}_2\text{-CH}_2\text{COOCH}_3$], δ 2.28 [t, ^3J 7.4 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.47 [t, ^3J 7.0 Hz, 4H, $\text{H}_3\text{C}(\text{CH}_2)_8\text{CH}_2\text{SCH}_2\text{-R}$], δ 3.64 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 14.37 (C-

19), δ 22.94 (C-18), δ 25.13 (C-3), δ 28.97 (C-7), δ 29.13, δ 29.22, δ 29.28, δ 29.53, δ 29.57, δ 29.81, δ 29.89, δ 29.99, δ 32.15 (C-17), δ 32.37, δ 32.46, δ 34.30 (C-2), δ 51.70 (-OCH₃), δ 174.49 (C1); MS (EI 70 eV) m/z 330 (M^+), 299 [(M - OCH₃)⁺], 189 [(M - (CH₂)₉CH₃)⁺], 173 [(M - (CH₂)₇COOCH₃)⁺], 159 [(M - (CH₂)₇COOCH₃)⁺], 157 [(M - S(CH₂)₉CH₃)⁺].

k. Methyl 9-Thiaicosanoate: $R_f = 0.12$ (2.5 % EtOAc/Hex); ¹H NMR (200 MHz) δ 0.85 [t, ³J 6.8 Hz, 3H, H₃CCH₂-R], δ 1.23, 1.28 [b, 22H, H₃C(CH₂)₈(CH₂)₂S(CH₂)₂-(CH₂)₃(CH₂)₂COOCH₃], δ 1.55 [m, 6H, H₃C(CH₂)₈CH₂CH₂SCH₂CH₂(CH₂)₃-CH₂CH₂COOCH₃], δ 2.28 [t, ³J 7.4 Hz, 2H, R-CH₂COOCH₃], δ 2.47 [t, ³J 7.2 Hz, 4H, H₃C(CH₂)₉CH₂SCH₂-R], δ 3.64 [s, 3H, -OCH₃]; ¹³C NMR (50.3 MHz) δ 14.39 (C-20), δ 22.96 (C-19), δ 25.13 (C-3), δ 28.99 (C-7), δ 29.15, δ 29.23, δ 29.28, δ 29.54, δ 29.60, δ 29.81, δ 29.88, δ 29.99, δ 32.17(C-18), δ 32.37, δ 32.44, δ 34.32 (C-2), δ 51.72 (-OCH₃), δ 174.51 (C1); MS (EI 70 eV) m/z 344 (M^+), 313 [(M - OCH₃)⁺], 189 [(M - (CH₂)₁₀CH₃)⁺], 187 [(M - (CH₂)₇COOCH₃)⁺], 157 [(M - S(CH₂)₁₀CH₃)⁺].

3.3 Oxidation of Substrates

The route described below is for the oxidation of methyl 9-thiapalmitate to methyl 9-sulfoxypalmitate. The procedure was repeated to produce all chain lengths from methyl 9-sulfoxydecanoate to methyl 9-sulfoxyeicosanoate.

3.3.1 Methyl 9-Sulfoxypalmitate

Methyl 9-thiapalmitate, (508.6 mg, 1.76 mmol), dissolved in CH_2Cl_2 , (4 mL), was transferred to a N_2 flushed, cooled, ($\sim 0^\circ\text{C}$, ice-water bath), 50 mL round bottom flask. *meta*-Chloroperoxybenzoic acid, (*m*-CBPA) (55%, 560.4 mg), dissolved in CH_2Cl_2 , (30 mL), was added dropwise to the reaction vessel via a plastic syringe, and the reaction mixture stirred for 30 minutes at $\sim 0^\circ\text{C}$. The solution warmed up to room temperature, over twenty minutes. The organic phase was washed with a freshly prepared aqueous base, (3×25 mL; 1.0 g NaOH, 8.0 g NaHCO_3 in 80 mL dH_2O). The aqueous phases from the washes were combined and extracted with CH_2Cl_2 , (3×30 mL). The organic phases were combined, dried over anhydrous Na_2SO_4 , concentrated in *vacuo*, and evaporated to constant weight under N_2 to give 503.0 mg of the sulfoxide as a yellowish-white solid. Purification by flash chromatography, (100 % EtOAc), yielded 343.6 mg (1.13 mmol, 64 %) yield of a colourless solid.

- a. Methyl 9-Sulfoxydecanoate:** liquid; R_f 0.09 (100 % EtOAc); ^1H NMR (200 MHz) δ 1.34 [b, 6H, $\text{CH}_3\text{S}(\text{CH}_2)_2(\text{CH}_2)_3(\text{CH}_2)_2\text{COOCH}_3$], δ 1.60 [m, 2H, $\text{RCH}_2\text{CH}_2\text{COOCH}_3$], δ 1.73 [m, 2H, $\text{H}_3\text{CSOCH}_2\text{CH}_2\text{-R}$], δ 2.28 [t, ^3J 7.5 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.53 [s, 3H, $\text{H}_3\text{CSO-R}$], δ 2.64 [d of d of d, ^3J 7.5, 7.0 Hz, ^2J 11.0 Hz, 2H, $\text{H}_3\text{CSOCH}_2\text{-R}$], δ 3.64 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 22.75 (C-7), δ 25.02 (C-3), δ 28.83, δ 29.06, δ 29.09, δ 34.22 (C-2), δ 38.80 (C-10), δ 51.75 ($-\text{OCH}_3$), δ 54.92 (C-8), δ 174.42 (C-1).
- b. Methyl 9-Sulfoxyundecanoate:** mp 26 – 28 °C; R_f 0.13 (100 % EtOAc); ^1H NMR (200 MHz) δ 1.31 [m, 9H, $\text{H}_3\text{CCH}_2\text{SO}(\text{CH}_2)_2(\text{CH}_2)_3(\text{CH}_2)_2\text{COOCH}_3$], δ 1.60 [m, 2H, $\text{R-CH}_2\text{CH}_2\text{COOCH}_3$], δ 1.74 [m, 2H, $\text{R-CH}_2(\text{CH}_2)_5\text{COOCH}_3$], δ 2.28 [t, ^3J 7.3 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.65 [m, 4H, $\text{H}_3\text{CCH}_2\text{SOCH}_2\text{-R}$], δ 3.64 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 7.05 (C-11), δ 22.79 (C-7), δ 25.04 (C-3), δ 28.93, δ 29.09, δ 34.23 (C-2), δ 45.89 (C-10), δ 51.72 ($-\text{OCH}_3$), δ 51.88 (C-8), δ 174.43 (C-1).
- c. Methyl 9-Sulfoxydodecanoate:** mp 28-30 °C; R_f 0.19 (100 % EtOAc); ^1H NMR (200 MHz) δ 1.06 [t, ^3J 7.3 Hz, 3H, $\text{H}_3\text{C-R}$], δ 1.32 [b, 6H, $\text{R-(CH}_2)_3(\text{CH}_2)_2\text{COOCH}_3$], δ 1.59 [m, 2H, $\text{R-CH}_2\text{CH}_2\text{COOCH}_3$], δ 1.77 [m, 4H, $\text{H}_3\text{CCH}_2\text{CH}_2\text{SOCH}_2\text{CH}_2(\text{CH}_2)_5\text{-COOCH}_3$], δ 2.28 [t, ^3J 7.5 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.60 [m, 4H, $\text{H}_3\text{CCH}_2\text{CH}_2\text{-SOCH}_2\text{R}$], δ 3.64 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 13.69 (C-12), δ 16.56 (C-11), δ 22.79 (C-7), δ 25.03 (C-3), δ 28.91, δ 29.08, δ 34.21 (C-2), δ 51.72 ($-\text{OCH}_3$), δ 52.65 (C-8), δ 54.65 (C-10), δ 174.42 (C-1).

- d. Methyl 9-Sulfoxytridecanoate:** mp 35 – 37 °C; R_f 0.21 (100 % EtOAc); ^1H NMR (200 MHz) δ 0.94 [t, 3J 7.2 Hz, 3H, $\text{H}_3\text{C-R}$], 1.34 [b, 8H, $\text{H}_3\text{CCH}_2(\text{CH}_2)_2\text{SO}-(\text{CH}_2)_2(\text{CH}_2)_3(\text{CH}_2)_2\text{COOCH}_3$], δ 1.60 [m, 2H, $\text{R-CH}_2\text{CH}_2\text{COOCH}_3$], δ 1.72 [m, 4H, $\text{H}_3\text{CCH}_2\text{CH}_2\text{CH}_2\text{SOCH}_2\text{CH}_2\text{-R}$], δ 2.28 [t, 3J 7.5 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.62 [m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_2\text{CH}_2\text{SOCH}_2\text{-R}$], δ 3.64 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (100.6 MHz) δ 13.71 (C-13), δ 22.11 (C-12), δ 22.56 (C-7), δ 24.63, δ 24.79, δ 28.69, δ 28.84, δ 28.86, δ 33.98 (C-2), δ 51.50 ($-\text{OCH}_3$), δ 52.21 (C-8), δ 52.38 (C-10), δ 174.19 (C-1).
- e. Methyl 9-Sulfoxytetradecanoate:** mp 41 – 43 °C; R_f 0.29 (100 % EtOAc); ^1H NMR (200 MHz) δ 0.89 [t, 3J 6.7 Hz, 3H, $\text{H}_3\text{C-R}$], δ 1.35 [b, 10H, $\text{H}_3\text{C}(\text{CH}_2)_2(\text{CH}_2)_2\text{SO}-(\text{CH}_2)_2(\text{CH}_2)_3(\text{CH}_2)\text{COOCH}_3$], δ 1.59 [m, 2H, $\text{R-CH}_2\text{CH}_2\text{COOCH}_3$], δ 1.74 [m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_2\text{CH}_2\text{CH}_2\text{SOCH}_2\text{CH}_2\text{-R}$], δ 2.28 [t, 3J 7.3 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.63 [m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_3\text{CH}_2\text{SOCH}_2\text{-R}$], δ 3.64 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 14.10 (C-14), δ 22.56 (C-13), δ 22.80 (C-7), δ 25.03 (C-3), δ 25.03, δ 28.92, δ 29.08, δ 31.26 (C-12), δ 34.23 (C-2), δ 51.75 ($-\text{OCH}_3$), δ 52.59 (C-8), δ 52.68 (C-10), δ 174.44 (C-1).
- f. Methyl 9-Sulfoxypentadecanoate:** mp 44 – 46 °C; R_f 0.31 (100 % EtOAc); ^1H NMR (200 MHz) δ 0.85 [t, 3J 6.8 Hz, 3H, $\text{H}_3\text{C-R}$], δ 1.31 [b, 12H, $\text{H}_3\text{C}(\text{CH}_2)_3(\text{CH}_2)_2\text{SO}-(\text{CH}_2)_2(\text{CH}_2)_3(\text{CH}_2)_2\text{COOCH}_3$], δ 1.58 [m, 2H, $\text{R-CH}_2\text{CH}_2\text{COOCH}_3$], δ 1.72 [m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_3\text{CH}_2\text{CH}_2\text{SOCH}_2\text{CH}_2\text{-R}$], δ 2.27 [t, 3J 7.4 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.60 [m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_4\text{CH}_2\text{SOCH}_2\text{-R}$], δ 3.63 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ

14.23 (C-15), δ 22.66 (C-14), δ 22.80 (C-7), δ 25.03 (C-3), δ 28.80, δ 28.92, δ 29.08, δ 31.62 (C-13), δ 34.22 (C-2), δ 51.74 (-OCH₃), δ 52.59 (C-8), δ 52.72 (C-10), δ 174.43 (C-1).

g. Methyl 9-Sulfoxyhexadecanoate: mp 53 – 55 °C; R_f 0.32 (100 % EtOAc); ¹H NMR (400 MHz) δ 0.82 [t, ³J 7.0 Hz, 3H, H₃C-R], δ 1.19, 1.25, 1.29, 1.40 [m of b, 14H, H₃C(CH₂)₄(CH₂)₂SO(CH₂)₂(CH₂)₃(CH₂)₂COOCH₃], δ 1.55 [m, 2H, R-CH₂CH₂-COOCH₃], δ 1.70 [m, 4H, H₃C(CH₂)₄CH₂CH₂SOCH₂CH₂-R], δ 2.24 [t, ³J 7.2 Hz, 2H, R-CH₂COOCH₃], δ 2.59 [m, 4H, H₃C(CH₂)₅CH₂SOCH₂-R], δ 3.60 [s, 3H, -OCH₃]; ¹³C NMR (100.6 MHz) δ 13.98 (C-16), δ 22.51 (C-15), δ 22.60 (C-7), δ 24.74 (C-3), δ 28.64, δ 28.67, δ 28.79, δ 28.81, δ 28.83, δ 31.51 (C-14), δ 33.93 (C-2), δ 51.42 (-OCH₃), δ 52.34 (C-8), δ 52.47 (C-10), δ 174.10 (C-1).

h. Methyl 9-Sulfoxyheptadecanoate: mp 56 – 58 °C; R_f 0.34 (100 % EtOAc); ¹H NMR (200 MHz) δ 0.85 [t, ³J 6.9 Hz, 3H, H₃C-R], δ 1.23, 1.31 [b of m, 16H, H₃C(CH₂)₅-(CH₂)₂SO(CH₂)₂(CH₂)₃(CH₂)₂COOCH₃], δ 1.59 [m, 2H, R-CH₂CH₂COOCH₃], δ 1.73 [m, 4H, H₃C(CH₂)₅CH₂CH₂SOCH₂CH₂-R], δ 2.28 [t, ³J 7.4 Hz, 2H, R-CH₂COOCH₃], δ 2.60 [m, 4H, H₃C(CH₂)₆CH₂SOCH₂-R], δ 3.64 [s, 3H, -OCH₃]; ¹³C NMR (50.3 MHz) δ 14.34 (C-17), δ 22.81 (C-16), δ 22.87 (C-7), δ 25.04 (C-3), δ 28.93, δ 29.10, δ 29.29, δ 29.43, δ 31.99 (C-15), δ 34.23 (C-2), δ 51.75 (-OCH₃), δ 52.60 (C-8), δ 52.73 (C-10), δ 174.43 (C-1).

- i. **Methyl 9-Sulfoxyoctadecanoate:** mp 60 – 62 °C; R_f 0.36 (100 % EtOAc); ^1H NMR (200 MHz) δ 0.85 [t, 3J 6.7 Hz, 3H, $\text{H}_3\text{C-R}$], δ 1.31 [b, 18H, $\text{H}_3\text{C}(\text{CH}_2)_6(\text{CH}_2)_2\text{SO}(\text{CH}_2)_2(\text{CH}_2)_3(\text{CH}_2)_2\text{COOCH}_3$], δ 1.59 [m, 2H, $\text{R-CH}_2\text{CH}_2\text{COOCH}_3$], δ 1.73 [m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_6\text{CH}_2\text{CH}_2\text{SOCH}_2\text{CH}_2\text{-R}$], δ 2.27 [t, 3J 7.4 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.60 [m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_7\text{CH}_2\text{SOCH}_2\text{-R}$], δ 3.63 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 14.37 (C-18), δ 22.81 (C-17), δ 22.87 (C-7), δ 22.90, δ 25.04 (C-3), δ 28.94, δ 29.10, δ 29.47, δ 29.59, δ 32.08 (C-16), δ 34.23 (C-2), δ 51.77 ($-\text{OCH}_3$), δ 52.60 (C-8), δ 52.74 (C-10), δ 174.45 (C-1).
- j. **Methyl 9-Sulfoxynonadecanoate:** mp 66 – 68 °C; R_f 0.36 (100 % EtOAc); ^1H NMR (200 MHz) δ 0.85 [t, 3J 6.9 Hz, 3H, $\text{H}_3\text{C-R}$], δ 1.23, 1.31 [b, 20H, $\text{H}_3\text{C}(\text{CH}_2)_7(\text{CH}_2)_2\text{-SO}(\text{CH}_2)_2(\text{CH}_2)_3(\text{CH}_2)_2\text{COOCH}_3$], δ 1.59 [m, 2H, $\text{R-CH}_2\text{CH}_2\text{COOCH}_3$], δ 1.73 [m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_7\text{CH}_2\text{CH}_2\text{SOCH}_2\text{CH}_2\text{-R}$], δ 2.28 [t, 3J 7.4 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.60 [m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_8\text{CH}_2\text{SOCH}_2\text{-R}$], δ 3.64 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 14.37 (C-19), δ 22.81 (C-7), δ 22.86 (C-18), δ 22.92 (C-7), δ 28.93, δ 29.01, δ 29.47, δ 29.51, δ 29.62, δ 29.75, δ 32.12 (C-17), 34.23 (C-2), δ 51.74 ($-\text{OCH}_3$), δ 52.61 (C-8), δ 52.75 (C-10), δ 174.42 (C-1).
- k. **Methyl 9-Sulfoxyeicosanoate:** mp 64 – 66 °C; R_f 0.37 (100 % EtOAc); ^1H NMR (200 MHz) δ 0.85 [t, 3J 7.0 Hz, 3H, $\text{H}_3\text{C-R}$], δ 1.24, 1.30 [b, 22H, $\text{H}_3\text{C}(\text{CH}_2)_8(\text{CH}_2)_2\text{-SO}(\text{CH}_2)_2(\text{CH}_2)_3(\text{CH}_2)_2\text{COOCH}_3$], δ 1.57 [m, 2H, $\text{R-CH}_2\text{CH}_2\text{COOCH}_3$], δ 1.73 [m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_8\text{CH}_2\text{CH}_2\text{SOCH}_2\text{CH}_2\text{-R}$], δ 2.28 [t, 3J 7.5 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ

2.61 [m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_9\text{CH}_2\text{SOCH}_2\text{-R}$], δ 3.64 [s, 3H, $-\text{OCH}_3$]; ^{13}C NMR (50.3 MHz) δ 14.39 (C-20), δ 22.82 (C-7), δ 22.88 (C-19), δ 22.94, δ 25.05 (C-3), δ 28.95, δ 29.13, δ 29.48, δ 29.58, δ 29.63, δ 29.81, δ 32.15 (C-18), δ 34.24 (C-2), δ 51.76 ($-\text{OCH}_3$), δ 52.62 (C-8), δ 52.75 (C-10), δ 174.21 (C-1).

3.3.2 Methyl 9-Sulfonylpalmitate

Methyl 9-thiopalmitate, (347.8 mg, 1.21 mmol), was oxidized with *m*-CPBA, (1.24g, 3.95 mmol), to form methyl 9-sulfonylpalmitate, (355.5 mg, 1.11 mmol), with a 92 % purified yield. A procedure similar to that of described in 3.3.1 was used for the formation of the product. Purification by flash chromatography, (25 % EtOAc/Hex), yielded 355.5 mg (1.11 mmol, 92 %), of a colourless solid.

mp 58 – 60 °C; R_f 0.25 (25 % EtOAc/Hex); ^1H NMR (400 MHz) δ 0.89 [t, 3J 6.9 Hz, 3H, $\text{H}_3\text{C-R}$], δ 1.33, 1.45 [m of b, 14H $\text{H}_3\text{C}(\text{CH}_2)_4(\text{CH}_2)_2\text{SO}_2(\text{CH}_2)_2(\text{CH}_2)_3(\text{CH}_2)\text{COOCH}_3$], δ 1.63 [p, 3J 7.4 Hz, 2H, $\text{R-CH}_2\text{CH}_2\text{COOCH}_3$], δ 1.83 [m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_4\text{CH}_2\text{CH}_2\text{SO}_2\text{-CH}_2\text{CH}_2\text{-R}$], δ 2.31 [t, 3J 7.5 Hz, 2H, $\text{R-CH}_2\text{COOCH}_3$], δ 2.94 [t, 3J 8.1 Hz, 4H, $\text{H}_3\text{C}(\text{CH}_2)_5\text{CH}_2\text{SO}_2\text{CH}_2\text{-R}$], δ 3.67 [s, 3H, (R-OOCH_3)]; ^{13}C NMR (100.6 MHz) δ 14.02 (C-16), δ 21.86, δ 21.97, δ 22.53, δ 24.75 (C-3), δ 28.33, δ 28.50, δ 28.73, δ 31.45, δ 33.95, δ 51.53 ($-\text{OCH}_3$), δ 52.65 (C-8), δ 52.81 (C-10), δ 174.19 (C-1).

3.4 Synthesis Of Phospholipids

The procedure described below is for the synthesis of DP(S)PC. The route was repeated in similar fashion to form DPPC, rDP(SO)PC, cDP(SO)PC, and DP(SO₂)PC.

3.4.1 9-Thiapalmitic Acid

Methyl 9-thiapalmitate, (278.9 mg, 9.67×10^{-4} mol), was mixed with 1.5 mL of 2 M KOH, (50 % dH₂O:EtOH). The mixture was heated in a water bath until a homogeneous solution resulted. The solution was allowed to cool to room temperature, acidified to pH 2 via 3 M H₂SO₄, and mixed with dH₂O, (15 mL). The solution became cloudy white upon addition of the acid. The aqueous mixture was extracted with CH₂Cl₂, (4 × 25 mL). The combined organic phases were dried over anhydrous Na₂SO₄, and concentrated in *vacuo*. The compound was chased with acetonitrile, (CH₃CN), and evaporated to give 244.3 mg, (8.90×10^{-4} mol, 92%), of the colourless solid.

a. 9-Thiapalmitic Acid: mp 37 – 39 °C; ¹H NMR (400 MHz) δ 0.87 [t, ³J 6.8 Hz, 3H, H₃C-R], δ 1.35 [m of b, 14H, H₃C(CH₂)₄(CH₂)₂S(CH₂)₂(CH₂)₃(CH₂)₂COOH], δ 1.60 [m, 6H, R-CH₂CH₂SCH₂CH₂(CH₂)₃CH₂(CH₂)₂COOH], δ 2.34 [t, ³J 7.5 Hz, 2H, R-CH₂COOH], δ 2.49 [t, ³J 7.4 Hz, 4H, H₃C(CH₂)₅CH₂SCH₂-R]; ¹³C NMR (100.6 MHz)

δ 14.09 (C-16), δ 22.63 (C-15), δ 24.61 (C-3), δ 28.72 (C-7), δ 28.87, δ 28.94, δ 29.64, δ 29.74, δ 31.76 (C-14), δ 32.13 (C-8), δ 32.22 (C-10), δ 33.97 (C-2), δ 179.83 (C-1).

b. Racemic 9-Sulfoxypalmitic Acid: mp 57 – 59 °C; ^1H NMR (400 MHz) δ 0.89 [t, ^3J 6.9 Hz, 3H, $\text{H}_3\text{C-R}$], δ 1.30, 1.36, 1.46 [m of b, 14H, $\text{H}_3\text{C}(\text{CH}_2)_4(\text{CH}_2)_2\text{SO}(\text{CH}_2)_2-(\text{CH}_2)_3(\text{CH}_2)_2\text{COOH}$], δ 1.63 [m, 2H, $\text{R-CH}_2\text{CH}_2\text{COOH}$], δ 1.77 [m, 4H, $\text{R-CH}_2\text{CH}_2\text{-SO}_2\text{CH}_2\text{CH}_2(\text{CH}_2)_5\text{COOH}$], δ 2.32 [t, ^3J 7.6 Hz, 2H, $\text{R-CH}_2\text{COOH}$], δ 2.70 [d of m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_5\text{CH}_2\text{SCH}_2\text{-R}$]; ^{13}C NMR (100.6 MHz) δ 14.05 (C-16), δ 22.55, δ 22.64, δ 24.66 (C-3), δ 28.61 (C-7), δ 28.78, δ 28.81, δ 28.86, δ 31.55, δ 34.01 (C-2), δ 51.96 (C-8), δ 52.13 (C-10), δ 177.89 (C-1).

c. Enantiomerically Enriched 9-Sulfoxypalmitic Acid: ^1H NMR (400 MHz) δ 0.89 [t, ^3J 6.8 Hz, 3H, $\text{H}_3\text{C-R}$], δ 1.29, 1.36, 1.46 [m of b, 14H, $\text{H}_3\text{C}(\text{CH}_2)_4(\text{CH}_2)_2\text{SO}(\text{CH}_2)_2-(\text{CH}_2)_3(\text{CH}_2)_2\text{COOH}$], δ 1.63 [m, 2H, $\text{R-CH}_2\text{CH}_2\text{COOH}$], δ 1.77 [m, 4H, $\text{R-CH}_2\text{CH}_2\text{SOCH}_2\text{CH}_2(\text{CH}_2)_5\text{COOH}$], δ 2.33 [t, ^3J 7.5 Hz, 2H, $\text{R-CH}_2\text{COOH}$], δ 2.70 [d of m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_5\text{CH}_2\text{SOCH}_2\text{-R}$]; ^{13}C NMR (100.6 MHz) δ 14.05 (C-16), δ 22.55, δ 22.64, δ 24.66 (C-3), δ 28.61 (C-7), δ 28.78, δ 28.81, δ 28.86, δ 31.55, δ 34.01 (C-2), δ 51.96 (C-8), δ 52.13 (C-10), δ 177.89 (C-1).

d. 9-Sulfonylpalmitic Acid: mp 83 – 85 °C; ^1H NMR (400 MHz) δ 0.89 [t, ^3J 6.9 Hz, 3H, $\text{H}_3\text{C-R}$], δ 1.29, 1.37, 1.45 [m of b, 14H $\text{H}_3\text{C}(\text{CH}_2)_4(\text{CH}_2)_2\text{SO}_2(\text{CH}_2)_2(\text{CH}_2)_3-(\text{CH}_2)\text{COOCH}_3$], δ 1.65 [m, 2H, $\text{R-CH}_2\text{CH}_2\text{COOCH}_3$], δ 1.83 [m, 4H, $\text{H}_3\text{C}(\text{CH}_2)_4-$

$\text{CH}_2\text{CH}_2\text{SO}_2\text{CH}_2\text{CH}_2\text{-R}$], δ 2.38 [t, ^3J 7.4 Hz, 2H, R- $\text{CH}_2\text{COOCH}_3$], δ 2.94 [b t, ^3J 8.1 Hz, 4H, $\text{H}_3\text{C}(\text{CH}_2)_5\text{CH}_2\text{SCH}_2\text{-R}$]; ^{13}C NMR (100.6 MHz) δ 14.00 (C-16), δ 21.85, δ 21.97, δ 22.52, δ 24.49, δ 28.32, δ 28.50, δ 28.65, δ 28.72, δ 28.73, δ 31.45, δ 33.77, δ 52.65 (C-8), δ 52.85 (C-10), δ 179.11 (C-1).

3.4.2 Dipalmitoyl-3-*sn*-phosphatidylcholine

L- α -Glycerophosphorylcholine, (1:1 cadmium chloride adduct from egg yolk) (101.6 mg, 2.31×10^{-4} mol), was dried under vacuum at 56 °C for 24 hours. 93.9 mg, (2.13×10^{-4} mol), was added to a flame dried, N_2 flushed 3-neck round bottom flask that contained dimethyl-4-aminopyridine, (DMAP) (55.1 mg, 4.51×10^{-4} mol), dicyclohexylcarbodiimide, (DCC) (161.9 mg, 7.85×10^{-4} mol), and palmitic acid, (279.2 mg, 1.09 mmol). CDCl_3 , (8 mL), purified via neutral alumina, was added to the reaction vessel, and the mixture stirred at room temperature under N_2 . The mixture was cloudy white after one hour of stirring, with some of the urea by-product collecting on the side of the flask. After 72 hours of stirring the mixture was passed through a pipette containing glass wool to remove the urea that had formed. Dipalmitoyl-3-*sn*-phosphatidylcholine, (DPPC), was purified using flash chromatography with an eluent gradient. The column was equilibrated with a 10:1 $\text{CHCl}_3/\text{MeOH}$, with a pressure of 30 kPa. After the

compound was deposited on the column, 10:1 $\text{CHCl}_3/\text{MeOH}$, (200 mL), was eluted, followed by 8:2 $\text{CHCl}_3/\text{MeOH}$, (100 mL), 7:3 $\text{CHCl}_3/\text{MeOH}$, (100 mL), 65:35 $\text{CHCl}_3/\text{MeOH}$, (100 mL), respectively. The column was completed using 65:35:4 $\text{CHCl}_3/\text{MeOH}:\text{dH}_2\text{O}$. The fractions were chased with CH_3CN to yield 77.7 mg, (1.06×10^{-4} mol, 46 %) of DPPC as a fluffy colourless solid.

a. Dipalmitoyl-3-*sn*-phosphatidylcholine: R_f 0.22 ; ^1H NMR (400 MHz) δ 0.88 [t, 3J 6.8 Hz, 6H, $(\text{R}-\text{CH}_3)_2$], δ 1.25 [s, 48H, $(\text{R} - (\text{CH}_2)_{12}\text{CH}_3)_2$], δ 1.57 [b, 4H, $(\text{R}'\text{-OOCCH}_2\text{CH}_2\text{-R})_2$], δ 2.28 [o t, 3J 7.4 Hz, 4H, $(\text{R}'\text{-OOCCH}_2\text{-R})_2$], δ 3.38 [s, 9H, $\text{R-N}(\text{CH}_3)_3$], δ 3.82 [b, 2H, $(\text{CH}_3)_3\text{NCH}_2\text{-R}$], δ 3.95 [b, 2H, $(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{-OPO}_2\text{OCH}_2\text{-R}$], δ 4.13 [d of d, 2J 12.0 Hz, 3J 7.3 Hz, 1H, $\text{R-H}_a\text{H}_b\text{COOC}(\text{CH}_2)_{14}\text{CH}_3$], δ 4.32 [m, 2H, $(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{-R}$], δ 4.40 [d of d, 2J 12.0 Hz, 3J 2.8 Hz, 1H, $\text{R-H}_a\text{H}_b\text{COOC}(\text{CH}_2)_{14}\text{CH}_3$], δ 5.20 [m, 1H, $(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{OPO}_2\text{OCH}_2\text{CH-R}$]; ^{13}C (100.6 MHz) δ 14.14 [$(\text{R}-\text{CH}_3)_2$], δ 22.71, δ 24.92, δ 25.00, δ 29.18, δ 29.21, δ 29.36, δ 29.39, δ 29.58, δ 29.70, δ 29.74, δ 31.95, δ 34.16 ($\text{R}'\text{-COOCH}_2\text{-R}$), δ 34.37 ($\text{R}'\text{-COOCH}_2\text{-R}$), δ 54.49 [$(\text{H}_3\text{C})_3\text{N-R}$], δ 59.30 [d, 2J 4.8 Hz, $(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{-R}$], δ 63.03 [$(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{OPO}_2\text{OCH}_2\text{CHRCH}_2\text{-R}$], δ 63.38 [d, 2J 5.0 Hz, $(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{OPO}_2\text{OCH}_2\text{-R}$], δ 66.44 [d, 3J 5.8 Hz, $(\text{CH}_3)_3\text{NCH}_2\text{-R}$], δ 70.58 [d, 3J 7.5 Hz, $(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{OPO}_2\text{OCH}_2\text{CH-R}$], δ 173.24 ($\text{R}'\text{-COOR}$), δ 173.59 ($\text{R}'\text{COOR}$); MS m/z 757 [$\text{M} + \text{Na}$] $^+$, 734 [M] $^+$.

- b. 9', 9''-Dithiapalmitoyl-3-*sn*-phosphatidylcholine:** R_f 0.22; ^1H NMR (400 MHz) δ 0.88 [t, 3J 6.9 Hz, 6H, (R-CH₃)₂], δ 1.25, 1.29, 1.37 [m of b, 28H, (R(CH₂)₂-(CH₂)₃(CH₂)₂S(CH₂)₂(CH₂)₄CH₃)₂], δ 1.57 [b, 4H, (R'-OOCCH₂CH₂-R)₂], δ 1.87 [b, 8H, (R-CH₂CH₂SCH₂CH₂(CH₂)₄CH₃)₂], δ 2.29 [o t, 3J 7.8 Hz, 4H, (R'-OOCCH₂-R)₂], δ 2.50 [t, 3J 7.4 Hz, 8H, R-CH₂SCH₂(CH₂)₅CH₃], δ 3.37 [s, 9H, R-N(CH₃)₃], δ 3.81 [b, 2H, (CH₃)₃NCH₂-R], δ 3.96 [b, 2H, (CH₃)₃N(CH₂)₂OPO₂OCH₂-R], δ 4.13 [d of d, 2J 12.0 Hz, 3J 7.2 Hz, 1H, R-H_aH_bCOOC(CH₂)₇S(CH₂)₆CH₃], δ 4.34 [m, 2H, (CH₃)₃NCH₂CH₂-R], δ 4.40 [d of d, 2J 12.0 Hz, 3J 2.8 Hz, 1H, R-H_aH_bCOOC-(CH₂)₇S(CH₂)₆CH₃], δ 5.21 [m, 1H, (CH₃)₃N(CH₂)₂OPO₂OCH₂CH-R]; ^{13}C (100.6 MHz) δ 14.10 [(R-CH₃)₂], δ 22.59, δ 24.89, δ 24.92, δ 28.80, δ 28.95, δ 29.03, δ 29.06, δ 29.68, δ 29.76, δ 31.78, δ 32.16 [(R-CH₂S(CH₂)₆CH₃)₂], δ 32.24 [(R-SCH₂(CH₂)₅CH₃)₂], δ 34.08 (R'-COOCH₂-R), δ 34.28 (R'-COOCH₂-R), δ 54.52 [(H₃C)₃N-R], δ 59.30 [d, 2J 4.9 Hz, (CH₃)₃N(CH₂)CH₂-R], δ 63.03 [(CH₃)₃N-(CH₂)₂OPO₂OCH₂CH-R], δ 63.42 [d, 2J 5.0 Hz, (CH₃)₃N(CH₂)₂OPO₂OCH₂-R], δ 66.47 [d, 3J 5.7 Hz, (CH₃)₃NCH₂-R], δ 70.59 [d, 3J 7.5 Hz, (CH₃)₃N(CH₂)₂O-PO₂OCH₂CH-R], δ 173.15 (R'-COOR), δ 173.50 (R'-COOR); MS m/z 793 [M + Na]⁺, 770 [M]⁺.
- c. 9',9''-(*R,S*)-Disulfoxypalmitoyl-3-*sn*-phosphatidylcholine:** R_f 0.22; ^1H NMR (400 MHz) δ 0.89 [t, 3J 6.8 Hz, 6H, (R-CH₃)₂], δ 1.29, 1.35, 1.45 [m of b, 28H, (R-(CH₂)₂(CH₂)₃(CH₂)₂SO(CH₂)₂(CH₂)₄CH₃)₂], δ 1.59 [b, 4H, (R'-OOCCH₂CH₂-R)₂], δ 1.76 [b, 6H, R-CH₂CH₂SOCH₂CH₂(CH₂)₄CH₃)₂], δ 2.30 [o t, 3J 7.5 Hz, 4H, (R'-

$\text{OOCCH}_2\text{-R}_2$], δ 2.67 [m, 8H, $\text{R-CH}_2\text{SOCH}_2(\text{CH}_2)_5\text{CH}_3$], δ 3.38 [s, 9H, $\text{R-N}(\text{CH}_3)_3$],
 δ 3.82 [b, 2H, $(\text{CH}_3)_3\text{NCH}_2\text{-R}$], δ 3.96 [b, 2H, $(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{OPO}_2\text{OCH}_2\text{-R}$], δ 4.13
[d of d, ^2J 12.1 Hz, ^3J 7.2 Hz, 1H, $\text{R-H}_a\text{H}_b\text{COOC}(\text{CH}_2)_7\text{SO}(\text{CH}_2)_6\text{CH}_3$], δ 4.32 [m,
2H, $(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{-R}$], δ 4.40 [d of d, ^2J 11.9 Hz, ^3J 2.5 Hz, 1H, $\text{R-H}_a\text{H}_b\text{COOC-}$
 $(\text{CH}_2)_7\text{SO}(\text{CH}_2)_6\text{CH}_3$], δ 5.21 [m, 1H, $(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{OPO}_2\text{OCH}_2\text{CH-R}$]; ^{13}C (100.6
MHz) δ 14.05 [$(\text{R-CH}_3)_2$], δ 22.56, δ 22.62, δ 22.65, δ 24.71, δ 24.76, δ 28.67, δ
28.70, δ 28.77, δ 28.81, δ 28.86, δ 28.90, δ 31.57, δ 31.60, δ 34.05 ($\text{R}'\text{-COOCH}_2\text{-R}$),
 δ 34.20 ($\text{R}'\text{-COOCH}_2\text{-R}$), δ 52.36 [$(\text{R-CH}_2\text{SO}(\text{CH}_2)_6\text{CH}_3)_2$], δ 52.57 [$(\text{R-}$
 $\text{SCH}_2(\text{CH}_2)_5\text{CH}_3)_2$], δ 54.51 [$(\text{H}_3\text{C})_3\text{N-R}$], δ 59.28 [d, ^2J 4.9 Hz, $(\text{CH}_3)_3\text{N}(\text{CH}_2)\text{CH}_2\text{-}$
 R], δ 63.00 [$(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{OPO}_2\text{OCH}_2\text{CHRCH}_2\text{-R}$], δ 63.38 [d, ^2J 5.2 Hz,
 $(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{OPO}_2\text{OCH}_2\text{-R}$], δ 66.49 [d, ^3J 6.0 Hz, $(\text{CH}_3)_3\text{NCH}_2\text{-R}$], δ 70.67 [d, ^3J
7.6 Hz, $(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{OPO}_2\text{OCH}_2\text{CH-R}$], δ 173.06 (R-COOR), δ 173.39 (R-
 COOR); MS m/z 825 [$\text{M} + \text{Na}$] $^+$, 802 [M] $^+$.

- d. 9'(R),9''(R)-Disulfoxypalmitoyl-3-sn-phosphatidylcholine:** R_f 0.22; ^1H NMR (400
MHz) δ 0.90 [t, ^3J 6.5 Hz, 6H, $(\text{R-CH}_3)_2$], δ 1.30, 1.37, 1.44 [m of b, 28H, $(\text{R-}$
 $(\text{CH}_2)_2(\text{CH}_2)_3(\text{CH}_2)_2\text{SO}(\text{CH}_2)_2(\text{CH}_2)_4\text{CH}_3)_2$], δ 1.60 [b, 4H, $(\text{R}'\text{-OOCCH}_2\text{CH}_2\text{-R})_2$], δ
1.76 [b, 6H, $(\text{R-CH}_2\text{CH}_2\text{SOCH}_2\text{CH}_2(\text{CH}_2)_4\text{CH}_3)_2$], δ 2.31 [o t, ^3J 7.9 Hz, 4H, $(\text{R}'\text{-}$
 $\text{OOCCH}_2\text{-R})_2$], δ 2.67 [m, 8H, $\text{R-CH}_2\text{SOCH}_2(\text{CH}_2)_5\text{CH}_3$], δ 3.37 [s, 9H, $\text{R-N}(\text{CH}_3)_3$],
 δ 3.81 [b, 2H, $(\text{CH}_3)_3\text{NCH}_2\text{-R}$], δ 3.97 [b, 2H, $(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{OPO}_2\text{OCH}_2\text{-R}$], δ 4.14
[d of d, ^2J 12.1 Hz, ^3J 7.0 Hz, 1H, $\text{R-H}_a\text{H}_b\text{COOC}(\text{CH}_2)_7\text{SO}(\text{CH}_2)_6\text{CH}_3$], δ 4.34 [m,
2H, $(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{-R}$], δ 4.41 [d of d, ^2J 12.0 Hz, ^3J 2.9 Hz, 1H, $\text{R-H}_a\text{H}_b\text{COOC-}$

$(\text{CH}_2)_7\text{SO}(\text{CH}_2)_6\text{CH}_3]$, δ 5.21 [m, 1H, $(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{OPO}_2\text{OCH}_2\text{CH-R}$]; ^{13}C (100.6 MHz) δ 14.04 [R-CH₃]₂, δ 22.54, δ 22.62, δ 22.64, δ 24.69, δ 24.73, δ 28.65, δ 28.68, δ 28.74, δ 28.79, δ 28.85, δ 28.88, δ 31.55, δ 33.97 (R'-COOCH₂-R), δ 34.17 (R'-COOCH₂-R), δ 52.33 [(R-CH₂SO(CH₂)₆CH₃)₂], δ 52.54 [(R-SOCH₂(CH₂)₅CH₃)₂], δ 54.56 [(H₃C)₃N-R], δ 59.25 [d, ^2J 4.9 Hz, $(\text{CH}_3)_3\text{N}(\text{CH}_2)\text{CH}_2\text{-R}$], δ 62.95 [(CH₃)₃N(CH₂)₂OPO₂OCH₂CH₂-R], δ 63.40 [d, ^2J 5.2 Hz, $(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{OPO}_2\text{OCH}_2\text{-R}$], δ 66.49 [d, ^3J 5.8 Hz, $(\text{CH}_3)_3\text{NCH}_2\text{-R}$], δ 70.61 [d, ^3J 7.3 Hz, $(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{OPO}_2\text{OCH}_2\text{CH-R}$], δ 173.07 (R-COOR), δ 173.42 (R-COOR); MS m/z 825 [M + Na]⁺, 802 [M]⁺.

- e. **9',9''-Disulfonylpalmitoyl-3-*sn*-phosphatidylcholine:** R_f 0.22; ^1H NMR (400 MHz) δ 0.88 [t, ^3J 7.0 Hz, 6H, (R-CH₃)₂], δ 1.29, 1.34, 1.43 [m of b, 28H, (R-(CH₂)₂(CH₂)₃(CH₂)₂-SO₂(CH₂)₂(CH₂)₄CH₃)₂], δ 1.60 [b, 4H, (R'-OOCCH₂CH₂-R)₂], δ 1.82 [b, 6H, (R-CH₂CH₂SO₂CH₂CH₂(CH₂)₄CH₃)₂], δ 2.30 [o t, ^3J 7.5 Hz, 4H, [(R'-OOCCH₂-R)₂], δ 2.94 [t, ^3J 7.6 Hz, 8H, R-CH₂SO₂CH₂(CH₂)₅CH₃], δ 3.37 [s, 9H, R-N(CH₃)₃], δ 3.80 [b, 2H, (CH₃)₃NCH₂ - R], δ 3.98 [b, 2H, (CH₃)₃N(CH₂)₂-OPO₂OCH₂-R], δ 4.13 [d of d, ^2J 11.8 Hz, ^3J 7.0 Hz, 1H, R-H_aH_bCOOC(CH₂)₇SO₂(CH₂)₆CH₃], δ 4.34 [m, 2H, (CH₃)₃NCH₂CH₂-R], δ 4.40 [d of d, ^3J 12.2 Hz, ^3J 3.0 Hz, 1H, R-H_aH_bCOOC(CH₂)₇SO₂(CH₂)₆CH₃], δ 5.21 [m, 1H, (CH₃)₃N-(CH₂)₂OPO₂OCH₂CH-R]; ^{13}C (100.6 MHz) δ 14.03 [R-CH₃]₂, δ 21.73, δ 21.76, δ 21.97, δ 22.53, δ 24.66, δ 24.70, δ 28.26, δ 28.29, δ 28.49, δ 28.61, δ 28.66, δ 28.73, δ 28.75, δ 31.46, δ 33.96 (R'-COOCH₂-R), δ 34.16 (R'-COOCH₂-R), δ 52.56, 52.57

[(R-CH₂SO₂(CH₂)₆CH₃)₂], δ 52.90, 52.83 [(R-SO₂CH₂(CH₂)₅CH₃)₂], δ 54.61 [(H₃C)₃N-R], δ 59.26 [d, ²J 4.9 Hz, (CH₃)₃N(CH₂)CH₂-R], δ 63.00 [(CH₃)₃N-(CH₂)₂OPO₂OCH₂CHRCH₂-R], δ 63.43 [d, ²J 5.0 Hz, (CH₃)₃N(CH₂)₂OPO₂OCH₂-R], δ 66.58 [d, ³J 5.8 Hz, (CH₃)₃NCH₂-R], δ 70.73 [d, ³J 7.6 Hz, (CH₃)₃N(CH₂)₂O-PO₂OCH₂CH-R], δ 173.04 (R'-COOR), δ 173.36 (R'-COOR); MS m/z 857 [M + Na]⁺, 834 [M]⁺.

3.5 Miscellaneous

3.5.1 Nitrosomethyl Urea

Methylamine, (61.212g, 1.97 mol), in a 40 % aqueous solution, was cooled in an ice bath, and mixed with concentrated HCl (250 mL) to ca. pH 1. Upon addition of HCl some white smoke came from the solution. Urea (300.42g, 5.00 mol) was added to the reaction vessel and the mixture refluxed for 2.5 hrs. The solution was allowed to cool to room temperature and mixed with sodium nitrite (155.50g, 2.25 mol). The solid was dissolved into the solution upon gentle heating. The homogeneous solution was introduced dropwise (over 30 min) into a 4L Erlenmeyer flask that contained a stirring mixture of ice (600 g) and concentrated H₂SO₄ (103.56 g). Upon addition, a yellow solid precipitated out. The nitrosomethyl urea was collected under vacuum, and was kept under suction until approximate dryness (127.14g 1.23 mol, 62%). The dry product was

tested via addition to a test tube containing an aqueous 50 % KOH solution (3 mL) and diethyl ether (15 mL). The compound started to bubble as it came in contact with basic solution and bubbled into the ethereal layer, turning the solution a golden yellow colour. The ethereal phase was mixed with stearic acid, and concentrated in *vacuo* to yield methyl stearate (100%). Nitrosomethyl urea was stored at 0 °C until further use.

3.5.2 Methyl *S*-(+)- α -Methoxyphenylethanoate

S- α -Methoxyphenylacetic acid (MPAA) (289.7 mg, 1.74 mmol) was mixed with anhydrous MeOH (20 mL), boron trifluoride diethyl etherate (4 mL), and refluxed for 2.5 hrs. The solution was allowed to cool to room temperature over 30 min. The solution was mixed with dH₂O (100 mL) and extracted with CH₂Cl₂ (3 $\times$ 50 mL). The combined organic phases were backwashed with saturated NaCl solution (50 mL), dried over Na₂SO₄, and concentrated in *vacuo* to give 255.4 mg (1.42 mmol, 81 %) of a colourless solid.

¹H NMR (200 MHz) δ 3.39 [s, 3H, R-CHOCH₃], δ 3.70 [s, 3H, R-COOCH₃], δ 4.76 [s, 1H, C₆H₅CH-RR'], δ 7.38 [m, 5H, C₆H₅-R]

3.6 Incubation Experiments

3.6.1 Yeast Δ^9 Desaturase

S. cerevisiae, wild strain #5288C, initially obtained as a liquid starter culture, Sue Patterson (Carleton University, Department of Biology), was streaked in a Petri dish and allowed to incubate at 30 °C over 48 hours. The resulting yeast colonies were stored at 4 °C. The medium for the Petri dishes were prepared as follows: A solution of BACTO™ peptone (20 g), BACTO™ yeast extract (10 g) and granulated agar (15 g) in 900 mL of deionized-distilled water (ddH₂O) was prepared in a 2 L Erlenmeyer flask. A solution of D-glucose (20 g) and ddH₂O (100 mL) was prepared in a 500 mL flask. The flasks were agitated at room temperature to ensure the compounds completely dissolved in the ddH₂O. The flasks were capped with sponge stoppers, to form a seal, wrapped in aluminium foil, and autoclaved. The two solutions were thoroughly mixed while they were still hot, and poured into the agar plates. All transfers of solutions after autoclaving were completed using sterile conditions. The resulting Petri dishes were allowed to cool to room temperature, for one hour. After the plates had solidified, the lids were placed on the Petri dishes, and the plates were wrapped with para-film. The Petri dishes were kept at 4 °C until 24 hours prior to their use.

S. cerevisiae was revived from a liquid culture for the majority of the experiments. The medium for the liquid cultures were prepared as follows: A solution of BACTO™ peptone (20 g), BACTO™ yeast extract (10 g) and ddH₂O (900 mL) was mixed in a 2 L Erlenmeyer flask. The solution was vigorously mixed at room temperature, capped with foam stoppers and aluminium foil, and was autoclaved. The solution was kept at 14 °C between uses, after one month the stock medium was

regenerated. The liquid culture of *S. cerevisiae* was prepared as follows: A solution of D-glucose (8 g) was mixed with ddH₂O (20 mL) in a 100 mL Erlenmeyer flask. 9 mL of medium was transferred to a 50 mL Erlenmeyer flask. The flasks were capped with foam stoppers, aluminium foil, and autoclaved. Upon cooling, the D-glucose solution (1 mL) was transferred into the medium. The 'starter' culture was inoculated with a small amount of yeast cells with a flamed wire loop, and allowed to incubate at 30 °C for 24 hrs. To maintain the liquid culture the procedure was repeated every seven days, using 4 drops of the previous liquid culture as a source for the yeast.

3.6.2 Enantiomerically Enriched Sulfoxidation Formation

The incubation of *ca.* 25 mg of substrate was prepared as follows: A solution of D-glucose (4 g) was mixed with ddH₂O (10 mL) in a 50 mL Erlenmeyer flask. 240 mL of the medium was transferred into a 1 L Erlenmeyer flask. The flasks were capped with foam plugs, aluminium foil, and were autoclaved along with pipettes wrapped in aluminium foil. The solutions were allowed to cool to room temperature. The D-glucose solution was added to the flask that contained the medium, and was mixed with 1.5 mL of yeast, from the starter culture. The substrate was mixed with 95 % EtOH (500 µL) and added to the flask. The vial that contained the substrate was rinsed with an additional 500 µL of EtOH and the culture was incubated at 30 °C for 24 hrs. The turbid solutions were centrifuged at 6000 rpm for 20 min at room temperature. The supernatant was decanted off to give a cell fresh weight of *ca.* 4-5 g.

3.6.3 Extraction of Enantiomerically Enriched Sulfoxides

The supernatant was acidified to *ca.* pH 3.8 at 0 °C, by adding glacial acetic acid, (*ca.* 3 mL), dropwise to the solution. The solution was then extracted with CHCl_3 (200 mL), this caused a large emulsion. The mixture was set aside, allowing the emulsion to subside. The organic phase as well as the remaining emulsion were set aside in a flask and the remaining aqueous phase was extracted with CHCl_3 (3×100 mL). The organic phases were combined along with any emulsions that also formed. The organic phase and emulsion mixture were separated in a separatory funnel, and there was little emulsion remaining. The organic phase was then extracted off and concentrated to yield a yellow coloured solid.

3.6.4 Methylation of Supernatant

Diethyl ether (15 mL) was added to a test tube that contained an aqueous 50 % KOH solution (3 mL) in an ice bath. Nitrosomethyl urea (350 mg, 3.40 mmol) was added to the test tube and as the compound came in contact with the base, the solution began to bubble and the ethereal solution turned yellow in colour, as it trapped diazomehtane. After the completion of the reaction the ethereal phase was dried over Na_2SO_4 and decanted into a RBF that contained the product of the supernatant extraction. The mixture was shaken at room temperature using a vortex and the resulting solution was concentrated in *vacuo* to yield a brownish-yellow solid. The crude solid was dissolved in EtOAc, passed through a pipette containing silica gel. The presence of sulfoxide was confirmed through TLC analysis (100 % EtOAc, $R_f = 0.22$) compared to a racemic standard. Purification via flash chromatography (100 % EtOAc) yielded 10 mg

of the enantiomerically enriched methyl 9-sulfoxyhexadecanoate as a slightly yellow coloured solid.

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